The organoleptic stability of British fresh pork sausages.

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THE ORGANOLEPTIC STABILITY OF BRITISH FRESH PORK SAUSAGES

submitted by Christopher Stephen Leads
for the degree of Ph.D.
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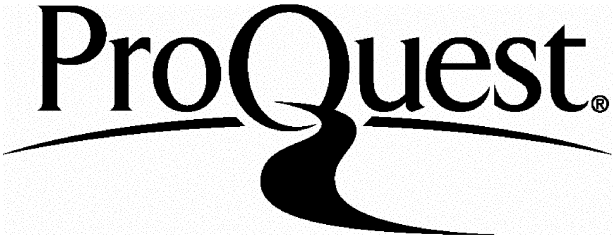
Christopher S. Leads



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SUMMARY

The relative organoleptic stability of the British fresh pork sausage among meat products has been shown in this study to be due more to a biochemical effect of SO_2 than to its antimicrobial activity (except for an inhibition of coliforms). Sulphiting doubled the storage life of film-wrapped sausages to four days at 20-22°C., prevented the formation of a strong cheese-like aroma, reduced the degree of colour change observed, prevented the accumulation of valeric acid, reduced the amounts of lactic acid, and a second unidentified acid, formed by ca. 50%, caused the accumulation, by preventing the metabolism, of C_{12}-C_{20} free fatty acids (but did not prevent the hydrolysis of glycerides), and limited the concomitant change in pH to 0.5 units.

The microbial association which developed under these conditions did not differ significantly in composition or size from that detected in unsulphited sausages, a finding which agreed with the results of Abbiss (1978) and Brown (1977).

Organoleptic change in the sulphited product, from acceptable to unacceptable, occurred when the microbial association at its surface exceeded 10^8 c.f.u./g. The

climax at ca. $10^{8.5}$ c.f.u./g. quickly followed, and the subsequent wave of post climax cell lysis was thought to contribute significantly to the quality changes perceived.

It was found that both the growth of the flora, and the pH change, were affected by glucose when SO_2 was present. A supply of glucose was envisaged as necessary to sustain the flora and maintain organoleptic stability, but a limitation to its availability was necessary to prevent too rapid microbial growth, an earlier climax, and, as a result, a shorter shelf-life. Of the sources of glucose studied, sausage rusk was found best to satisfy these requirements cheaply. In addition it contributed a "baked cereal" component to the aroma of the sausage, and this was regarded as a characteristic and desirable feature.

INTRODUCTION

The British fresh sausage, a perishable commodity having a shelf life of about 12 days at 0-4°C, and about 5 days at room temperature (ca. 20-22°C), accounts for upwards of 10% of the meat consumed in the United Kingdom. It has been estimated that the annual turnover is about £260 million. This very large market is serviced by specialist manufacturers, and local butchers. The former bring to bear the normal trappings of marketing (advertising, display, incentives etc.) in attempting to increase the sale of their products. In the final analysis, of course, appearance and flavour will most probably determine whether or not housewives continue to purchase a particular brand. As the commodity has a limited shelf-life, the production capacity of large manufacturers is geared to meet peak demand, and the distribution network to the daily delivery of sausages to the retail outlets. Both contribute to costs. Savings could be achieved if factories could even-out prodction and deliveries could be staggered.

Although the perishable nature of sausages is well known, there appears to have been little work done on the course of spoilage - the trade tends still to use the general term, sour, for sausages which have spoiled -

or those factors which the consumer considers to be the attributes of a fresh as opposed to a stale saŭsage. The present investigation was concerned with these two features and an attempt was made to correlate change in organoleptic properties with microbial activity.

Not only does this thesis discuss the results of studies dealing with microbial growth and organoleptic properties, it does provide information about ingredients, marketing etc. so that the overall problem of manufacture and selling of a perishable commodity is put in perspective.

THE MARKET

This section is concerned with the size of the market and the factors that appear to influence the consumer's choice of sausage.

In 1977, 262,000 tons (55% by weight pork, 45% non-pork) of fresh sausages were consumed (Table 1). This represented an expenditure of £259 million at retail selling prices (Table 1). Consumption nationally increased in 1977 over the previous three years, reflecting the slower increase in sausage prices compared to those of meat and other manufactured foods (Fig. 1). The consumption of meat other than pork fell during the period 1975-77 (Fig. 2) whereas sausage consumption increased by approximately 6% in 1977 after having remained constant for the preceding two years, and having declined by 39,000 tons per annum from a peak of 282,000 tons in the period 1968-75 (Table 1).

The price of sausages increased on average from 18.2p/lb to 43.8p/lb in the period 1970-77 (Table 1). The average differential between pork and non-pork sausages in the same period fell from 2.8p to 1.9p/lb (1970-73), and then rose to 3.4p (1977), thus accentuating the purchase of the cheaper non-pork varieties. The price of sausages is such, however, that they retain an overall

majority (57.3%) of the butcher's"small-goods" market valued in 1975 at £361 million (Fig. 3).

Sausages are sold from two main outlets; butchers shops and supermarkets. The former often manufacture and sell sausages on the same premises, and sell them in any desired weight or number. A few supermarkets also follow this policy, but the majority get their sausages in film-wrapped 1lb packs from large manufacturers. This appears to be a growing trend.

In 1976 the ratio of the value of sales was estimated to be butchers 53% : supermarkets 47%, but the ratio of weights sold was 38% : 62% (Anon. 1978). Another, more detailed, analysis (Anon. 1975) showed the division to be butchers 35% : supermarkets 48% : freezer-centres 7% : others 10%. As butchers are unable to compete with the price of the mass-produced sausage, they now tend to make either a high quality, or a very cheap low quality, product. The same survey (Anon. 1975) also suggested that there is very little difference between the main products of the major sausage manufacturers because of the public's preference for a bland, cheap, consistent commodity.

Socio-economic status and geographical location also influence purchasing preference. Managerial and professional classes prefer to purchase from supermarkets,

whilst their opposite socio-economic group, old age pensioners and the unemployed, buy from the local butcher. A butcher's sausage is preferred in the North, a supermarket's in the South (Table 2). Regional differences in preference between pork and non-pork sausages have also been demonstrated (Fig. 4).

As with all products, the consumer's acceptance of sausages is subjective. In the case of sausages, two stages can be recognised. In the shop the appearance and texture of the product will be assessed by the consumer. Of course the choice of wrapping material, label design and colour, the shape and arrangement of the sausages as well as the lighting and the display will probably influence this assessment (Birch, Brennan and Parker 1977). The aroma and taste of the cooked product will provide the final assessment, but this will be done in the home. Little, if anything,is known about the factors that influence this latter assessment. This study sought to identify some of the factors involved, and to assess the contribution of micro-organisms to change in the organoleptic properties of sausages.

TABLE 1

Domestic expenditure on, and consumption of, fresh sausages in the United Kingdom, 1970 - 1977 (Anon. 1977)

Parameter	Sausage	1970	1971	1972	1973	1974	1975	1976	1977
Domestic consumption of fresh sausage in thousands of tons	Pork	178	178	157	147	148	134	139	145
	Non Pork	104	100	111	111	117	109	109	117
	Total	282	278	268	258	265	243	248	262
Division of domestic sausage market by type as % of total consumption	Pork	63		59		56		56	55
	Non Pork	37		41		44		44	45
	Total	100		100		100		100	100
Annual National Average household food prices for sausages (pence/lb), and price differential (Pork - Non Pork)	Pork	19.58	21.00	22.73	27.19	30.93	35.82	41.26	45.49
	Non Pork	16.79	18.57	20.61	25.24	28.74	32.65	37.85	42.12
	Difference	+2.79	+2.43	+2.12	+1.95	+2.19	+3.16	+3.41	+3.37
Domestic expenditure on fresh sausages (£million)	Pork	78	83	80	90	102	107	128	145
	Non Pork	39	42	51	63	75	88	93	111
	Total	117	125	131	153	177	195	221	259

TABLE 2

Table of results of survey by Mintel/B.M.R.B. into factors influencing choice of retail outlet

Criterion	Classification	Retail Outlet (% Sales)		
		Butcher	Supermarket	Other
Social Group+	A/B	42	57	6
	C1	53	43	10
	C2	50	45	6
	D/E	63	40	3
Age	Under 35	48	48	6
	35-54	54	44	8
	Over 54	61	43	3
Family Status	with children	50	46	8
	without children	58	43	3
Location	South	38	57	8
	Midland	56	45	5
	North	66	34	3
	All	54	44	6

+ A/B : Management/Professional
C1 : Skilled Workers
C2 : Unskilled Workers
D/E : Unemployed/O.A.P.s

Fig 1 Wholesale Price Index changes.

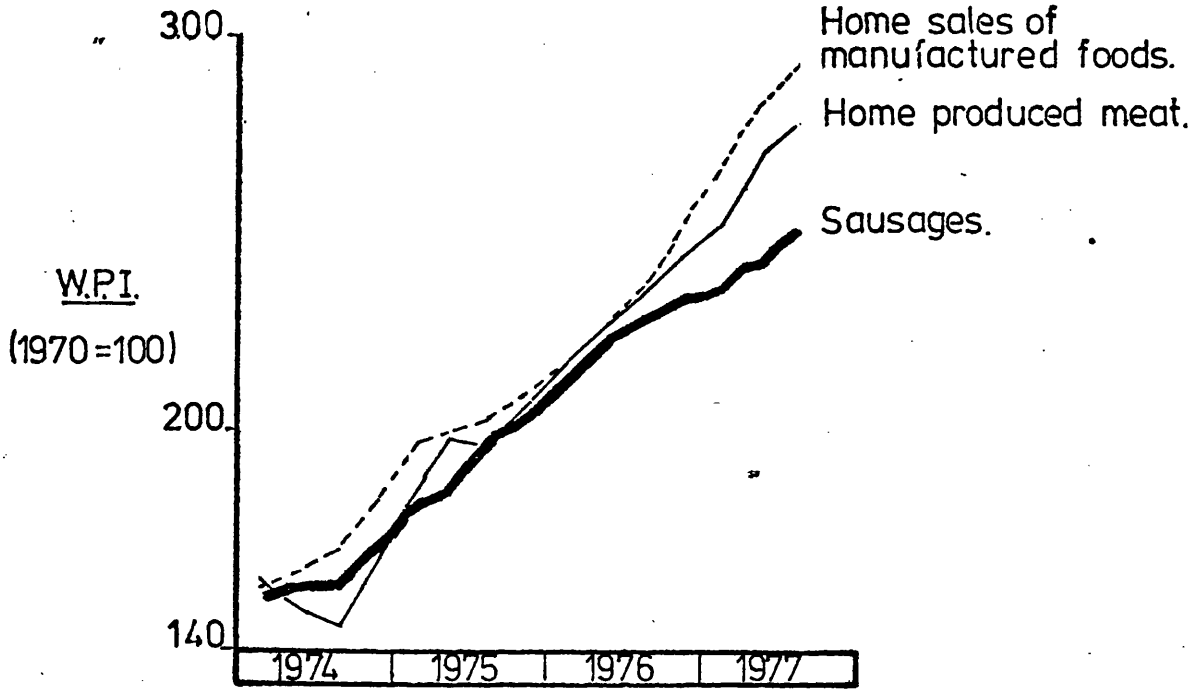


Fig 2 Comparitive consumption trends.

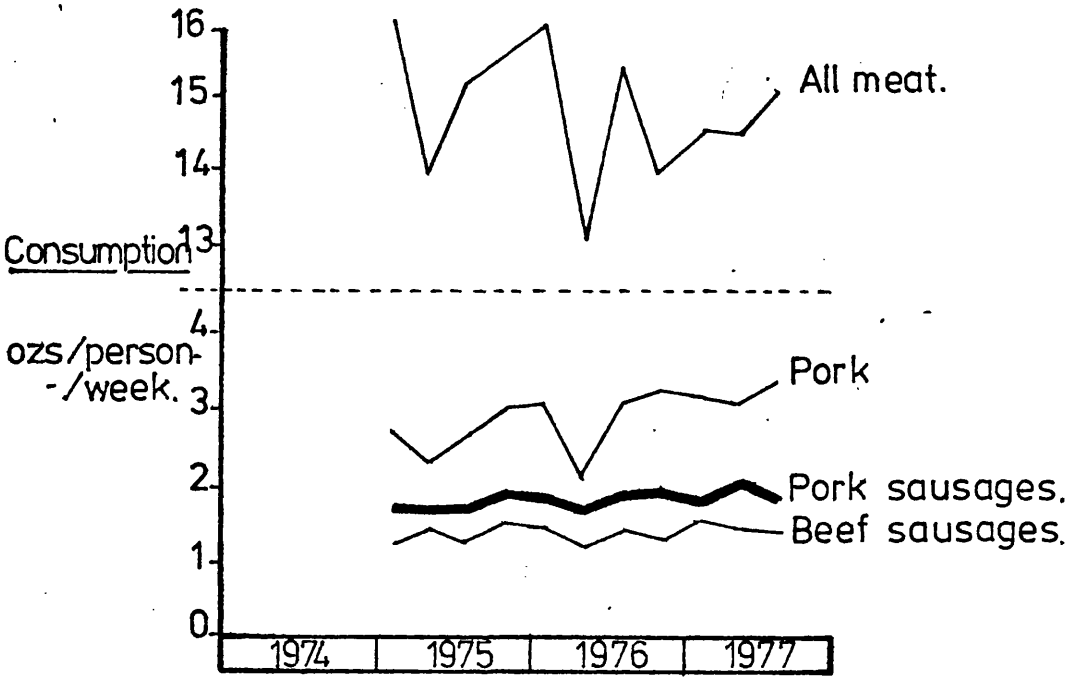
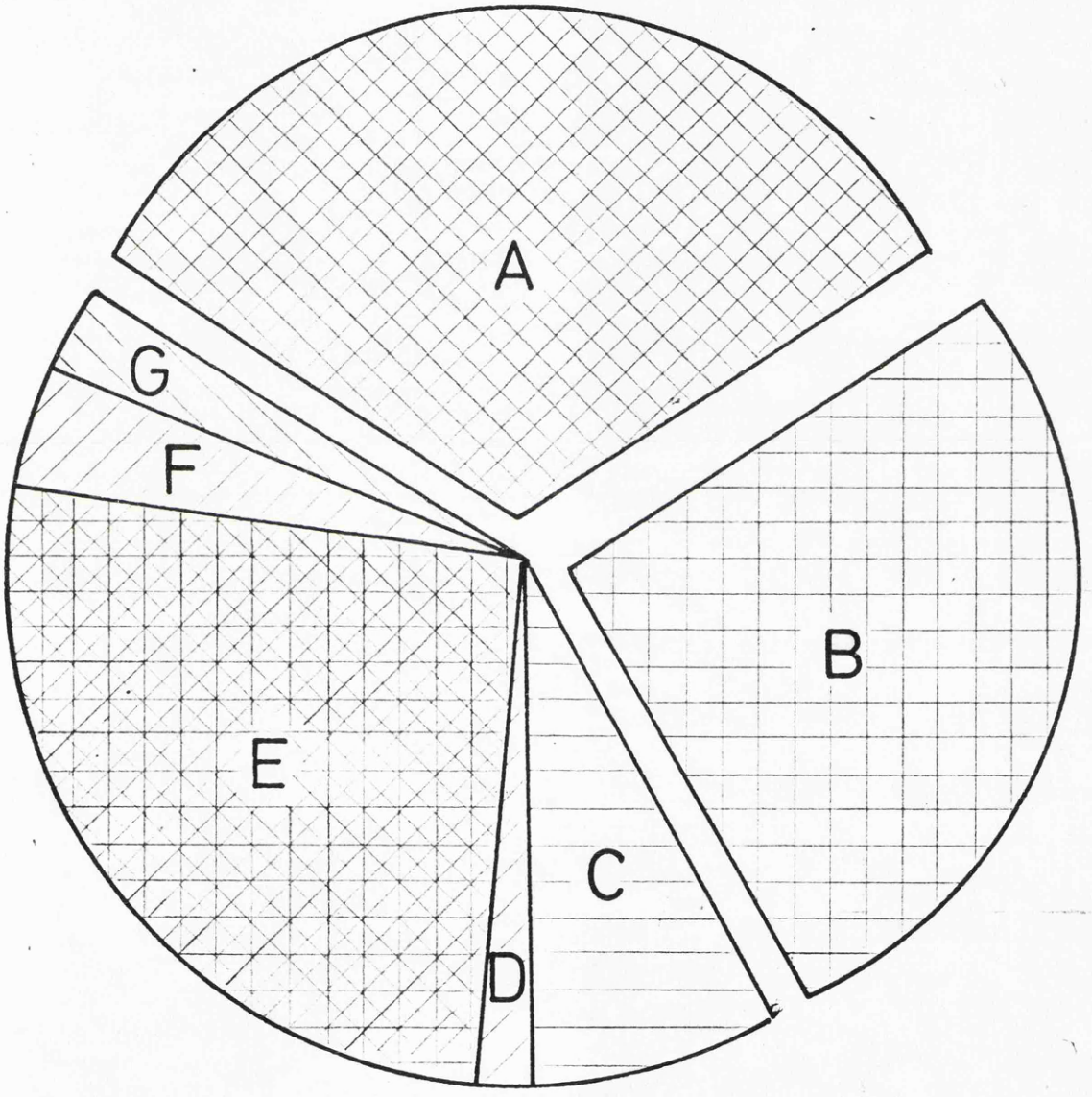


Fig 3: The division of the 1975 "small-goods" market, by weight.

[ref: "The market for sausages & pies" Mintel ltd. 1975]

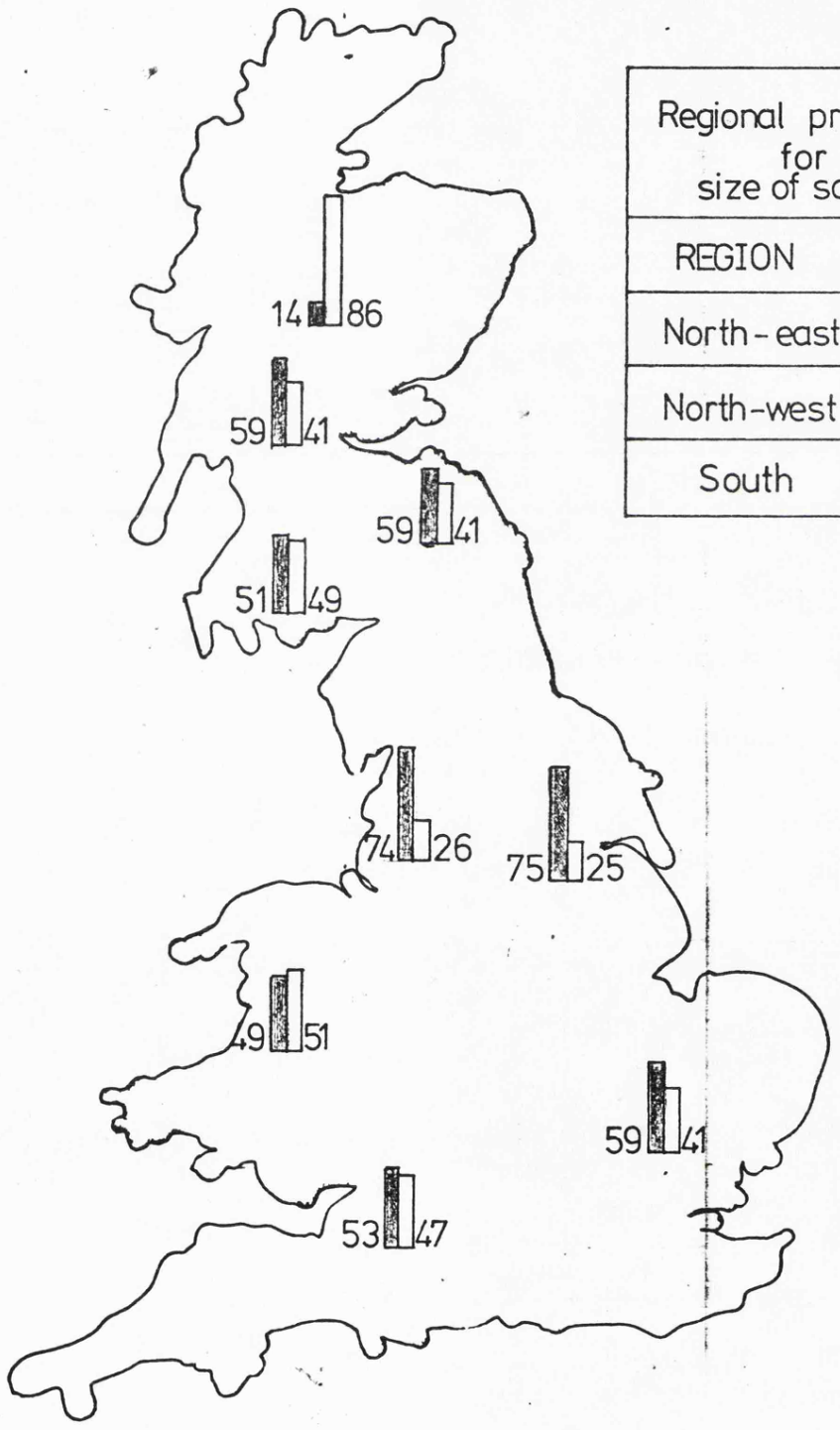


Sector.	Commodity.	Tonnage.	Value.	Market share.
A.	Pork sausages	142,000	£120m.	31·4%
B.	Other sausages	117,000	£ 81m.	25·9%
C.	Pork Pies.	34,000	£ 28m.	7·5%
D.	Veal & Ham Pies.	8,000	£ 7m.	1·8%
E.	Hot-meat Pies.	120,000	£ 100m.	24·0%
F.	Sausage rolls	18,000	£ 15m	6·6%
G.	Pasties.	12,000	£ 10m	2·7%

Fig. 4

REGIONAL PREFERENCES FOR PORK (■) & NON-PORK (□) SAUSAGES, AS PERCENTAGE OF WEEKLY CONSUMPTION.

(ozs. / person.)



Regional preference for size of sausage		
REGION	8/lb	16/lb
North-east	45%	55%
North-west	40%	60%
South	50%	50%

THE COMPOSITION AND MANUFACTURE OF FRESH SAUSAGES

Over the years the manufacturers of sausages in the United Kingdom have become dependent on suppliers of ingredients such as seasonings, breadcrumbs etc. Competition between these suppliers has led to a relatively high standard of material being offered to the manufacturers. The manufacturer is forced to change the formulae of his products as the prices of raw materials change. In addition, he is dependent upon relatively crude machines and practices for the preparation of sausages. This section reviews the range of ingredients used in sausage manufacture and attempts to identify the major contributions which they make to the initial organoleptic quality of the product.

COMPOSITION

An ideal fresh Pork sausage will have the composition 65% meat, 20% water, 12.5% sausage rusk, and 2.5% seasoning. A variety of formulations are, in actual fact, observed (Table 6).

The product comprises of a mixture of comminuted meat, rusk, water, flavourings (mainly from herbs and spices), a preservative (SO_2) and other additives (e.g. colours, emulsifiers and antioxidants), extruded under pressure

into the tube ('casing') obtained from the submucosa of the small intenstines of pigs or sheep. Alternatively, two 'synthetic' casings may be used; either tubes formed from reprecipitated collagen into which the sausage meat mixture is extruded, or a film of coagulated meat protein formed on the surface of the sausage meat mixture by exposure to boiling water after extrusion (the 'skinless' sausage).

The total meat, lean meat, and named meat contents of fresh sausage is legally defined (Fig. 5). In Pork sausages these requirements are satisfied by combining lean cuts from the porcine carcase (e.g. 'belly' and 'hand' - Fig. 5) with shoulder meat, and fats such as back fat from which the rind has been removed (derinded). Beef sausages also contain derinded pork back fat, pork hand and shoulder, beef neck, and sometimes breast of lamb or cheap mutton (according to availability and cost). Pig jowls ('face meat') and skin ('rinds'), the latter either boiled and minced, or emulsified, may be used in cheaper sausage varieties.

Water is the second largest ingredient by weight, and sausage rusk the third. Rusk is a crumb produced by milling a dried, slightly leavened bread made from a dough of soft wheat flour (Appendix 1), salt, water, and ammonium bicarbonate. It absorbs twice its weight in water, without crumbling or forming a paste. This

feature is important to the final texture of the sausage because it absorbs and binds the 20% (w/w) of water which is normally added to the product.

The sausage seasoning, now generally used as a pre-blended, pre-weighed ingredient, is usually added at a level of 1.5 - 3.0% (w/w). In addition to herbs and spices (or extracts of their flavour components) it will probably contain antioxidants (Vitamins C or K), colour holding additives (niacin or niacinamide), polyphosphates (to bind added water), soya protein mixtures (to emulsify and bind excess fat) and sodium sulphite or metabisulphite, the source of the permitted preservative, sulphur dioxide. Salt, an important flavour component in sausages, is present at 0.8 - 1.2% (w/w) of the raw product. As it is usually added with the other seasoning components, it is often the main ingredient of the seasoning blend.

The seasoning blend is intended principally to contribute that flavour which each manufacturer regards as 'unique'. In addition it fulfills a secondary role of controlling certain physical characteristics of the raw and cooked sausage (e.g. colour holding, 'drip-loss' of water during cold storage, and shrinkage during cooking - Plate 1). The permitted maximum initial level of sulphur dioxide (450 parts/10^6) is also achieved by

including its precursors - sodium sulphite or meta-bisulphite - in the seasoning, and standardising the latter's usage rate for a given weight of sausage meat mixture so that it conforms with legal requirements.

The colour of some sausage is determined in part by the addition of red pigments. These may be either of natural origin (e.g. Carmine) or synthetic (e.g. Red 2G). Conditions within the sausage limit the number which are suitable. Red 2G, erythrosine, tartrazine (a yellow pigment) and carmine have been found by experience to be the best suited to resist the bleaching effects of SO_2, micro-organisms, and meat enzymes in the sausage, and sunlight (to which sausages on display are exposed).

MANUFACTURING TECHNIQUES

There are two different methods; 'mince and mix', and bowl chopping.

(i) Mince and Mix

Mince and mix is a derivative of the traditional method for manufacturing sausages. The meats are minced (through a plate with holes of a diameter between 3 and 5 millimetres) and then combined with water, seasoning and rusk in a mixing vessel. The mixture is filled into casings using hydraulically-powered piston fillers, and

'linked' (twisted into individual sausage lengths) either mechanically or manually. 'Batching' (eight or sixteen to the pound) and wrapping of the finished sausages may be done either mechanically or manually.

(ii) Bowl Chopping

The bowl chopping method is based on the Continental procedure for producing Wiener-type (i.e. Frankfurter) sausages. The bowl-chopping machine comprises a torus-shaped circular bowl with a set of knives mounted radially. Both the bowl and knives are powered, and rotate; the contents of the bowl may therefore be continuously passed through the knives. The degree of comminution the meat receives may therefore be controlled.

Meat, previously butchered to ca. 4" pieces, is added to the chopper bowl with the seasoning and half of the water, and comminuted as described above. When the meat texture becomes sticky and the water has been absorbed, the rusk and remaining water are added, and the mixture blended and comminuted until the desired texture is achieved.

Both processes are labour intensive, and are liable to errors, both human and mechanical. Freshly produced sausages often fail to achieve minimum quality standards

as set by the manufacturers. Examples of the more common errors encountered in the sausage manufacturing industry are given (Table 3).

TABLE 3

Some errors commonly encountered during sausage manufacture, and their assigned causes

Fault	Assigned Cause	Reason
Accelerated loss of colour of sausages	Meat of poor micro-biological quality	Meat with unacceptable TVC (ca. 10^7/g) will give initial TVC in sausages of ca. 10^6/g. Sausages of this initial quality have a shorter shelf-life and discolour rapidly.
	Poor control of temperature during manufacture (Target 4°C)	(a) Some sausage ingredients have exothermic heats of solution (e.g. rusk), causing the temperature of the sausage mixture to rise when water is added. At higher temperatures, microbial growth will accelerate. (b) Pork rind emulsions are often prepared hot. Inadequate chilling can result in rapid microbial growth to ca. 10^9/g. (Appendix III)
	Manufacturing error	A weighing or mixing error during seasoning manufacture (e.g. the omission of preservative or antioxidant) can result in poor colour holding of the final sausage.

TABLE 3 (Continuation 1)

Fault	Assigned cause	Reason
Accelerated loss of colour of sausages (cont/.)	Contamination with nitrite	Cross-contamination from nitrited products (e.g. Saveloys or pork pies) results in rapid loss of colour. 0.2 p.p.m. $NaNO_2$ is sufficient to cause this effect. (R. Scott pers. comm.)
	Nitrate in process water.	Nitrates, present in process water (as a result of leaching from soils dressed with fertilizer) can be reduced by micro-organisms to nitrite, resulting in above effect.
	Enzyme activity	Some soya bean products are enzymatically active, and their use in sausage can result in loss of colour. (Author's own experience)
Pink or red spots	Dye particles or high local dye concentrations	(a) Coarse particles of dye, which dissolve slowly, causing highly localised dye concentrations. (b) Dye adsorbed to oleoresins dispersed on salt. Dye is therefore locally concentrated.

TABLE 3 (Continuation 2)

Fault	Assigned Cause	Reason
White Spots	Pimpling	Particles of underhydrated rusk - caused by a fault of the rusk itself, or insufficient water in the recipe - will be visible as white spots, and can be felt as hard particles.
	Minced rind	Coarsely chopped rind, being white and reflective, will be visible against the background colour of the sausage mixture.
	White spot	Rancid fat, with a high peroxide value, will cause localised deoxygenation of meat pigments, causing particles of grey.
Uneven stripes of colour	Pressure marks	Restricted to wrapped sausages, or sausages kept in contact with each other. Effect due to differences in state of oxygenation of myoglobin (caused by high TVC, rancid fats or peroxides) due to O_2 limitation created between sausage surfaces in contact compared to those exposed to air.

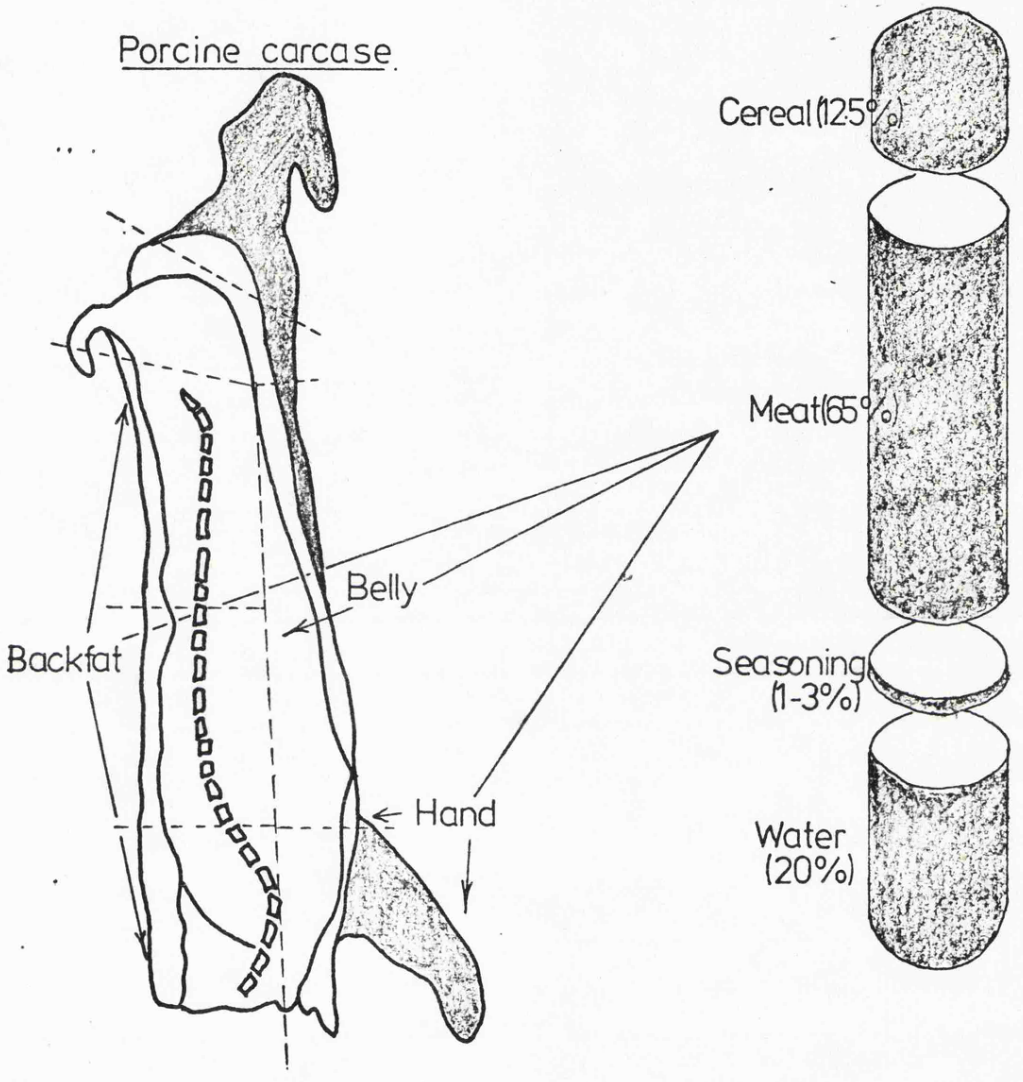
TABLE 3 (Continuation 3)

Fault	Assigned cause	Reason
Poor water retention. Drip-loss on chilling.	Poorly balanced recipe	Excessive water not properly bound, due to the use of poor quality meats, or inadequate quantities of polyphosphates.
Uneven sausage length. (Automatic fillers only)	Variations in rheological properties of mix	Variations in rheological qualities of mixes will cause automatic filling ('twist-linkers') machines, which fill volumetrically, to extrude varying amounts of mixture. Commonly caused by water-binding variations due to dust levels in rusk being too high.

Plate 1

The effect of polyphosphates (present in A, B, C, & D) on the retention of water during cooking.

Fig5 The origin of the meat cuts used in pork sausages the proportions of the other ingredients, & legal requirements.



LEGISLATION

1. The sausage & other meat product regulations (1967).

		Total	Named	Lean
% Minimum meats:	Pork sausages:	65	80	50
	Beef sausages:	50	50	50

2. The preservatives in foods regulations (1975).

Permitted preservative: Sulphur dioxide (SO_2)

Maximum level permitted: $450p/10^6$.

PRESENTATION

Sausages displayed for purchase are presented to the customer in one of two ways, depending upon the manufacturer. Butchers tend to display their product linked in bundles of four and suspended from a hook, or piled loosely on a tray. In both instances, the quantity of sausage manufactured is limited to the amount which can be sold in a day, and therefore wrapping to impede moisture loss is regarded as unnecessary. In contrast, large sausage manufacturers need to protect their product against moisture loss during storage and distribution prior to display, and their sausages are therefore packaged in a film, usually by weight, in units of one pound.

In addition to conserving moisture, the packaging film used to wrap sausages also serves other functions; it allows gaseous exchange to occur, whilst preventing the loss of volatile flavour components, and it also provides a surface for printing the manufacturer's name, the identity of the product, and any necessary legal declarations.

THE SENSORY EVALUATION OF FOODS

Little is known about scientific quality assessment of sausages. Such tests as are performed tend to fall into two categories; assessment of flavour by tasting the cooked product immediately after manufacture, and assessment of shelf life by the observation of colour holding. Sensory measurement of time dependent changes is rarely, if ever, used as a measure of quality.

A person's acceptance of a food depends upon the image mentally constructed from information from the many senses. Six have been described (Harper 1977): sight, hearing, smell, taste, touch and kinaesthesis - the ability to discriminate variations in force and leverage (e.g. bite). They are evaluated in the sequence: appearance, odour, texture and consistency, taste and aroma (von Sydow 1971). Decisions made during evaluation involve recognition, discrimination, quantification, pattern recognition and finally preference (Harper 1977). Stored information from previous experiences is used in this process.

At its simplest, the decision made may be either to like (accept) or dislike (not accept). In practice the recall of stored intermediate sensory information used to arrive at the above conclusion is now recognised to play a part in a more subtle assessment of the organoleptic properties of a food. The information derived from the assessment

process may be either qualitative or quantitative. The former is descriptive and usually based upon an accepted vocabulary, derived either by agreement prior to the evaluation (von Sydow, Anderson et al. 1970, von Sydow 1971, Mecredy, Sonneman and Lehman 1974), or selected from the vernacular as, for example, with baking (Cornford 1977) and tea-tasting (Theobald 1977).

Quantitative assessments rate each sensory attribute in numerical, or numerically related terms, and are extensions of qualitative methods.

The information contained in sensory impressions, being biological in origin, is limited by the extremes of sensitivity of the senses themselves. The lower limit is the threshold concentration, below which stimuli cannot be perceived, and the upper limit, the saturation concentration above which discrimination cannot be made (Amerine, Pangbourne and Roessler 1965). Between the limits, all perceivable stimuli can be positioned and, or, quantified.

A continuous gradient of sensation (a continuum) is thought to occupy the sensory space between the limits, although the brain registers 'difference' in terms of a series of discreet intervals of stimulus intensity. Sensitivity is then a measure of the size of these intervals. Psychophysics has defined a unit of

sensitivity as the 'just-noticeable-difference' (j.n.d.) between two stimuli (Stevens 1957).

To compensate for the natural variations in sensitivity between individuals, the j.n.d. is based upon an assumed probability rather than a finite difference. For two samples of food, for example, evaulated for a common sensory attribute (e.g. saltiness), the point of subjective equality is the trial in which half the assessors can detect a difference and half cannot, an outcome regarded as no better than chance. The differential threshold (j.n.d.) is then, by convention, the trial in which 75% of comparisons are assessed as different (Stevens 1957).

Using this technique it has been demonstrated that the spatial relationships of j.n.d.'s in continua differ according to the sense used, intensity of stimulation, and interpretation of sensory information (i.e. as quantitative or qualitative results).

Qualitative information is judged by a process of substitution, and the perceived j.n.d.'s are usually linearly distributed throughout the continuum. The stimulus is judged by position rather than intensity (e.g. as with pitch).

Quantitative judgements are additive, and the spatial

distribution of their j.n.d.'s is non-linear. J.n.d. size has been found to vary according to the intensity of the stimulus, and the sense used, and is regarded as physiological limitation of the senses themselves rather than an error in interpretation. A result of this assymetry is the recognition that such sources of information are liable to two inherent errors; a 'time-order' effect, and a 'hysteresis' phenomenon (Stevens 1957). The former is a systematic bias towards assessing the second sample in a pair greater or better than the first. The effect is strongest when the samples are equivalent and dilute, and is considered due to the j.n.d. being most sensitive at sensations near to the threshold value. In short, where no difference exists, the mind can deceive itself into creating one (Stevens 1957).

The latter, hysteresis, is a similar phenomenon. A scale of j.n.d.s produced by incrementally increasing the intensity of the stimulus will not have the same form if the order is reversed. For accuracy, therefore, a consistency of direction of assessment must be maintained.

Although the j.n.d. is a useful measure of difference, the amount of experimental work required to establish its magnitude and distribution characteristics for a given sensation limits its usefulness to research applications in psychophysics. An understanding of its

characteristics is important in the interpretation of experimental results.

For measurements of change concerning preference rather than, or in addition to, difference, a scale of reference may be used instead. Three types of scale have been devised for the estimation of change in sensory continua; rating, scoring and magnitude estimation (Harper 1977).

A variety of rating and scoring scales have been described (Amerine, Pangbourne and Roessler 1965). In the majority of cases they are used in conjunction with an internal standard whose qualities are known and agreed. A common special case is the hedonic scale, in which a sensory characteristic is categorised on a scale of superlatives. The number of categories offered varies; although numbers from 3 to 15 have been described the most efficient number for rating without excessive confusion is probably 9 (Gardner 1960). Because a fixed number of categories are offered, this type of scale is self-limiting; any of the range of sensations experienced can be described by one of the categories offered.

In contrast, magnitude estimation is an open system of scaling limited only by the physiological limits to the senses previously described. It is based upon judgements of the ratio of stimulus intensities between paired samples, and is a derivative of the j.n.d. technique (Stevens 1957,

Stevens and Gallantine 1958).

The types of scale mentioned are all used to describe characteristics of the same kind of sensory experience, and they have an exponential relationship (Eisler 1962, Eisler 1963). Similar comparisons have been made between sensory information and results obtained from analytical instruments; an example is the measurement and comparison of aroma and flavour componenents in foodstuffs (Powers and Keith 1968, Powers 1968, Vuatez and Raymond 1971, von Sydow 1972). Again the relationship is exponential, derived either from an algebraically defined lined of best fit (Ekman 1961) or assumed initially to be a derivative of Stevens' law (Stevens 1957) and assessed for statistical robustness by regression analysis (Persson and von Sydow 1974).

The consideration of the use of analytical instruments for the measurement of aroma and flavour components stems from a desire to standardise organoleptic assessment, eliminate bias, and accelerate work. The best results will probably be obtained from a combination of both instrumental and sensory techniques. The advantages of the former are low running cost, consistency of operation, and rapid response; disadvantageously they are expensive to purchase, require a great deal of developement before they can be used, and yield limited information. A taste panel, in contrast, will tend to be inconsistent in operation, liable to intrinsic bias, and expensive to run. It

is, however, flexible, retrainable, and mobile.

For the purpose of this study, an untrained panel was used to evaluate and score stored sausages for appearance, aroma and flavour. Category ratings, supported in the case of aroma by a second, non-compartmentalised graph-rating technique, was chosen as the scoring method. Time order errors and hysteresis were minimised by randomising samples and using a 'double-blind' sample presentation technique. The experiments were designed to allow the estimation of time dependent changes in the three stimuli studied, and to produce information suitable for subsequent statistical analysis.

THE MICROBIOLOGY OF FRESH SAUSAGES

A feature of the manufacture of sausages is that, once the ingredients have been combined, mixed, and stuffed into casings, they are not subjected to further processing. Although it is probable that the cellular organisation of the main ingredient, meat, is destroyed by comminution, the enzymes are merely released (Brown 1977, Abbiss 1978) as well as the microflora which had developed in the interval between slaughter and use (Hurst 1972).

The initial studies (Dyett and Shelley 1966) showed that a Gram positive flora was dominant in stored sausages containing sulphite. It is now established that 'factory' sausages contain an association of Microbacterium thermosphactum, Pseudomonas spp., species of yeasts, and Lactobacillus spp. (Brown 1977, Abbiss 1978). Souring, the name used by the trade to describe sausage spoilage, has been attributed to the physiological activities of the members of the association, but there is little evidence to support such a contention. Indeed, the slight change in pH during storage would not seem to be sufficient alone to cause gross organoleptic change, particularly as lactic acid, a bland substance, is considered to be a principal product of microbial action. One aim of this thesis was to correlate changes in the size and composition

of the microbial association with specific organoleptic changes which could be constrained as 'spoilage'.

Contamination, both in numbers and types of micro-organisms, of the individual ingredients of sausages varies as does their contribution of micro-organisms to the initial flora (Tables 4 and 5). Meat, 50-65% of the sausage by weight, makes the greatest contribution. Because the live animal has a range of natural defences against invasion by micro-organisms, commercially important contamination of meat occurs after slaughter. Its nature and extent depend upon three features of its production; how the live animal was treated just before slaughter, the temperature of storage of the carcase after slaughter, and how skilfully the meat was butchered (Ayres 1955, Spencer 1966).

Insanitary lairage of animals, and distressing conditions (e.g. overcrowding) can reduce the microbiological quality of the resulting carcase by causing an increase in the level of skin contamination, and a decrease in the quality of the meat by reducing its pH (Elmossalami and Wassef 1971, Gardner 1966). Cross-contamination of the meat, and penetration of muscle blocks if skinning and evisceration are carelessly executed, can easily result.

Regardless of the care exercised during slaughtering, commercial abbatoirs produce meat contaminated with between 10^3 and 10^6 bacteria per gramme. The rate of growth, and composition, of the contamination depends subsequently upon the temperature and duration of storage. Refrigeration, the most common method, favours the election of Pseudomonas spp. as the dominant contaminants (Ingram and Dainty 1966). As the temperature of the storage environment increases, the dominance of this species is gradually reduced; by ca. 20°C it has been replaced by genera from the Enterobacteriaceae, and at physiological temperatures (ca. 37°C) by Clostridium spp.

Microbacterium thermosphactum, the dominant organism of wrapped pork sausages (Dowdell and Board 1968, Hurst 1972, Brown 1977, Abbiss 1978) is a Gram positive pleomorphic rod (Davidson, Mobbs and Stubbs 1968) first isolated from American breakfast sausage (Szulsbacher and Maclean 1953), and subsequently from lamb and chicken joints (Barlow and Kitchell 1966), sulphited minced pork (Hurst 1972) and beef (Roth and Clarke 1975). Its morphological similarity to Lactobacillus may have caused confusion in earlier studies (Dyett and Shelley 1966), and its true distribution was not known until the development of a selective medium (Gardner 1966). Although it is now known that the organism is a common contaminant of meat, its natural niche has not been identified.

Psychrotrophic Pseudomonas spp. are usually the second most numerous group of organisms in sausages (Brown 1977, Abbiss 1978). Their initial contribution to the association is quite low (ca. 10^3/g) but rises as the sausage is stored (Hurst 1972, Brown 1977, Abbiss 1978). Their overall contribution appears greater in sausages stored under refrigeration than at room temperature (Brown 1977, Abbiss 1978). In cold stores, poor rotation of chilled stocks can result in some carcasses developing large populations of Pseudomonas spp., and the subsequent use of meat from this source in sausages can produce co-dominance of the sausage flora by Pseudomonas spp. and Microbacterium thermosphactum (Hurst 1972).

The yeast and Lactobacillus populations are invariably smaller than those of the dominant organisms; they rarely exceed 1% of the total population during the growth phase of the association (Hurst 1972, Brown 1977, Abbiss 1978). Despite being numerically in a minority, the contribution of yeasts to spoilage is significant because of their greater mass, cell volume and biochemical activity. Yeast dominated sausages have been observed occasionally during experimental work (Brown 1977), and the products of at least one large manufacturer are known to be dominated by them.

The organisms mentioned above are simply those that grow out from the heterogenous flora present at the time of sausage manufacture (Table 5). Of those that failed to grow appreciably, only Micrococcus spp. can be recovered without recourse to enrichment methods during the product's shelf life (Brown 1977, Abbiss 1978). Bacillus spp., present in quite large numbers on herbs and spices (Julseth and Deibel 1974, and Appendix 2), in soya protein extracts and rind emulsions (Appendix 3), and sausage casings (Riha and Solberg 1970) appear not to survive the manufacturing process in significant numbers. As yet, there is no evidence with which to account for their failure to survive let alone grow. In contrast, the Coliform population present on raw meat is known to be prevented from growing in sausages by the presence of SO_2 (Christiansen 1953, Hurst 1972, Brown 1977, Abbiss 1978). Moulds (Aspergillus spp. Penicillium spp.) do not occur in pork sausages unless heavily contaminated raw materials have been used, the sausages have been stored at high temperatures (ca. 30°C) or have aged well in excess of their shelf life.

Storage temperature is a selective pressure for Microbacterium thermosphactum: the organism has a limiting temperature for growth of 25°C (Gardner 1966), and this corresponds to the maximum temperature at which sausages are usually stored. At temperatures approaching those

of commercial chillers (4°C) it remains dominant but its proportion of the total flora is reduced (Brown 1977, Abbiss 1978). It has been shown that an increase in the level of atmospheric carbon dioxide favours the development of Microbacterium thermosphactum on beef, except in circumstances where Lactobacillus spp. are similarly stimulated (Roth and Clarke 1977). Under these conditions, the growth of Pseudomonas spp. is inhibited (Roth and Clarke 1975, Corlett 1975). In contrast an increased level of atmospheric oxygen does not stimulate the Pseudomonas population nor impede the development of Microbacterium thermosphactum or Lactobacillus (Corlett 1975).

The above studies were done on 'shrink-wrapped' beef joints. A film with similar gaseous-diffusion properties is used to wrap sausages, and has three principal functions; to hold the pack together, thus making the product easier to handle, to provide a surface for printed information, and to conserve the moisture content of the sausages whilst permitting gaseous exchange. A range of suitable films have been described (Selby 1961, Anderton and Barnes 1967), but thin-guage polyethylene is most generally favoured in the industry. Although it will allow free passage of gases between the sausages and the environment, the tight wrapping of sausages with film probably impedes free gaseous exchange within the pack because the individual links are compressed. If there is any residual respiratory

activity in the meat in the sausage, elevated levels of carbon dioxide will probably result within the pack. Under such conditions the dominance of Microbacterium thermosphactum should be assured, although the effect of the preservative, SO_2, is not known.

TABLE 4

Types of micro-organisms found on the main ingredients of pork sausages

Ingredient	Micro-organisms present	Reference or source of information
MEAT (from chiller)	Microbacterium thermosphactum Pseudomonas spp. Coliforms Lactobacillus spp.	Ingram & Dainty (1971) Barlow & Kitchen (1966) Hurst (1972) Dowdell & Board (1968)
SAUSAGE RUSK	Micrococcus spp. Aspergillus spp. Penicillium spp. Yeasts	T. Lucas & Co. Ltd.
ADDED WATER	None present in significant numbers	
SALT	None present in significant numbers	British Salt Ltd.
SOYA PROTEINS	Bacillus spp. Yeasts	T. Lucas & Co. Ltd.
POLYPHOSPHATES	None present in significant numbers	Albright & Wilson Ltd.

TABLE 4 (Continuation 1)

Ingredient	Micro-organisms present	Reference or source of information
HERBS & SPICES (fumigated)	Bacillus spp. Clostridium spp.	Julseth & Diebel 1974 Hadlock & Touré 1973 T. Lucas & Co. Ltd.
ESSENTIAL OILS	None present in significant numbers	Various suppliers
CASINGS	Bacillus spp.	Riha & Solberg 1970

TABLE 5

The overall contributions of the main sausage ingredients to the total flora
(see Table 4)

Ingredient	Level of contamination	Proportion of recipe	Contribution
MEAT (from chiller)	10^3-10^5/g. 10^6 max.	65-80%	6.5×10^2 - 8.0×10^4/g.
SAUSAGE RUSK	10^2-5×10^3/g.	12.5-17.0%	125-1.7×10^2/g
WATER	<10/ccm.	ca. 20%	<10/g.
SALT	<10/g	0.8-1.5%	<10/g.
SOYA PROTEIN	10^2-10^4/g.	ca. 0.3%	3×10^2/g. max.
POLYPHOSPHATES	<10/g.	ca. 0.3%-1%	<10/g.
HERBS & SPICES	10^2-10^6/g.	0.1-0.2%	10-2×10^4/g.
ESSENTIAL OILS	<10/g.	<0.1%	<10/g.
CASINGS	10^1-10^6/g.	ca. 1%	<10-10^4/g.

TABLE 6

Examples of analyses of the [+]composition of commercially available pork sausages

Sample	(1)% Fat	(2) % Lean Meat	(3) % Total Meat (1+2)	% Cereal	% Added water	% Ash
A	17.8	57.3	75.1	12.2	11.1	1.4
B	15.9	49.6	65.5	13.5	18.9	1.6
C	29.3	39.9	69.2	14.5	14.2	1.5
D	32.6	44.7	77.3	6.7	14.2	1.6
E	19.4	35.9	55.3	21.8	20.4	1.8
F	19.9	51.0	70.9	11.4	15.5	1.6

[+]analysis of samples routinely evaluated by the Analytical Services Section T. Lucas & Co. Ltd.

CHEMICAL COMPOSITION AND CHANGE

The legal constraints controlling the meat content, named meat content and lean to fat ratio (Fig. 5) define minimum requirements. Within these limits a diverse range of sausage formulations can result (Table 6) because of the opinions of individual manufacturers as to what makes a good sausage.

The analyses in Table 6 confirm that the proportions of the ingredients approximate to that desired (Fig. 5). Their individual contributions to the overall balance of the pork sausage (in terms of protein, fat, carbohydrate and water) are given in Table 7.

Belly, and shoulder or hand, pork cuts are used in sausages together with derinded pork backfat and perinephric fat if an emulsifier such as soya protein is used. Freshly killed meat has a potential for fairly rapid autolysis at 37°C, but under conditions of chilled storage (4°C) the period required for significant change in the muscle structure to occur with the release of significant amounts of amino acids, exceeds the normal storage life of pork destined for sausages (Locker 1960). Although the activity of cathepsins post-rigor may cause the release of small quantities of free amino nitrogen, its accumulation does not make it an alternative energy source to the fat and carbohydrate present (Ingram and Dainty 1971).

Pork fat, at 15-30% of the sausage, represents a large resource of carbon and energy. Amongst animal fats pork is richest (Table 8) in polyunsaturated fatty acids (Hilditch 1956). The overall ratio of saturated to unsaturated free fatty acids esterified as triglycerides in pork depot fat is 35 : 65 (Sink et al. 1962), although the position of the component acids at individual sites in the porcine carcase will vary according to gender, age and, particularly, live weight. Up to 130lbs (i.e. ca. 120 days) unsaturated fatty acids are preferentially deposited at deep sites on the carcase (i.e. perinephric and deep sub-cutaneous) rather than near the surface. Above 130lbs perinephric fat becomes more saturated, as do deep sub-cutaneous fats. In general terms, therefore, as pigs age, their body fats become more saturated.

The cereal component - sausage rusk - is mainly carbohydrate, with small quantities of protein and water, but no fat (Table 7). The wheat starch granules are a pool of metabolisable carbon, and their degradation to glucose by porcine amylases has been demonstrated (Abbiss 1978). Glucose is probably a primary substrate for the microbial association. An alternative might be glycerol (Brown 1977), released from glycerides by glycerol-ester hydrolases. Although this class of enzyme is highly substrate specific, Microbacterium thermosphactum is reported to synthesize a glycerol-ester hydrolase with a

specificity for carbon chains of less than 12, for individual free fatty acids (Collins-Thompson et al. 1971). Aerobic yeasts isolated from sausages are thought to produce them also (Dowdell and Board 1971). Their hydrolytic activity will also release any acids from glycerides, and these may subsequently be catabolised. β-Oxidation is the most common pathway in animal tissues, although α-oxidation by bacteria can occur in contaminated tissues (Morris 1970).

In addition to the principal ingredients described above, a range of minor ingredients are added which have specific effects on the sausage. The osmolarity of the sausage is increased by the presence of soluble salts of inorganic compounds (e.g. sodium chloride, sodium tripolyphosphates, sodium hexametaphosphate). Its pH is buffered from ca. 5.3 (post-rigor meat) to ca. 6.5 (Hurst 1972, Brown 1977, Abbiss 1978), and SO_2 is present at an initial concentration of 450 mgs./kg. sausage. The redox potential may be influenced by the addition of the antioxidants, vitamins C and K, in order to impede the rate of reduction of myoglobin to its less attractively coloured reduction product, metmyoglobin. Thiamine (from the flour used in rusk production and from the pork, a rich source) is also present (McCance and Widdowson 1960).

The sum of these individual features is an environment within the sausage which is biochemically totally different

to that of the main ingredient, raw meat. Apart from the changes in the carbon to nitrogen ratio, the raw sausage mixture, compared to meat, does not have structural integrity or cellular compartmentalisation; these have been destroyed during the bowl chopping procedure. In addition cutting up the meat tissue, and mixing the ingredients, beats in air. Normal barriers to diffusion are destroyed and oxygen gradients are disturbed.

In total there occurs a sudden change from a series of ordered environments (meat, sausage rusk etc.) to a single highly disordered one. This sudden change may contribute much to the observed stasis in microbial growth in the sausage for the first 24 hours after manufacture (Hurst 1972, Brown 1977, Abbiss 1978). To date there has been an emphasis on the cardinal role of SO_2 in this stasis.

It is reported that spoilage of the freshly manufactured sausage, as measured by certain biochemical parameters, follows predictable trends. The pH, ca. 6.5 initially, slowly decreases to ca. pH 6.0 during the span of the shelf life (Hurst 1972, Brown 1977, Abbiss 1978) and the rate of decrease is greater in sausage surfaces exposed to the air (Brown 1977, Abbiss 1978). Sulphur dioxide exists in two states within the sausage - bound and free - and loss of the latter occurs more rapidly at the surfaces exposed to air. The rate of loss approximates to first-

order kinetics (Brown 1977).

Lactic acid is said to accumulate (Dowdell and Board 1968) perhaps via the catabolism of glucose (Abbiss 1978), or glycerol (Brown 1977). The low volatility of lactic acid and its low dissociation constant (Mahler and Cordes 1971) suggest that it is not a single cause of the objectionable odour of spoiled sausage. This is probably the result of other changes.

The aroma and flavour of meat has been attributed to the fat (Sink 1973); lean meats are said to have a similar meat-like flavour, but the fat component the characteristic taste. Changes in the aroma of spoiling pork sausages may be due to deterioration of the fat, perhaps by oxidation (Brown 1977). α- and β-oxidation will generate short chain free fatty acids from hydrolysed glycerides; many are volatile and have objectionable aromas. Alternatively, auto-oxidation of unsaturated fats (which constitute a large proportion of pork fat) will cause hydroperoxidation and subsequent scission of the fatty acid chain. An accumulation of odiferous carbonyl compounds, soluble in fat, will result (Sink 1973). It is reported that SO_2 can initiate and promote hydroperoxide formation (Brown 1977). Some micro-organisms also cause this transformation, including groups associated with pork sausage flora (Micrococcus spp., Pseudomonas spp., and Microbacterium thermosphactum) (Goldman 1955, Finnerty 1962, Smith and Alford 1968). Ferric ions and haem, when present,

are reported to act as rate accelerators (Wills 1965).

The production of glucose from starch by amylases has been studied (Abbiss 1978). Pork sausage fat, and its degradation (if any) has not been investigated apart from studies of its susceptibility to SO_2 mediated auto-oxidation (Brown 1977). Because it constitutes ca. 30% of a pork sausage, and is labile, it may be a major feature of sausage aroma and spoilage. A gas chromatographic study was done to explore this hypothesis.

The metabolic oxidation of fats requires thiamine as a cofactor for cocarboxylase function (Mahler and Cordes 1971). Pork, a rich source of thiamine, normally contains ca. 10 $p/10^6$ of the vitamin, ca. 10-20 times that of other meats (McCance and Widdowson 1960). The flour used in rusk production is also fortified with thiamine (McCance and Widdowson 1960), the quantities of which are not greatly affected by bread and biscuit baking processes (Farrer 1955). It is, however, rapidly destroyed in aqueous solution in the presence of SO_2 (Schroeter 1966). It has been proposed (Brown 1977) that this might be a possible effect of SO_2 in pork sausages, and further evidence for this was sought.

TABLE 7

Analysis of fresh pork sausage and its principal ingredients (McCance & Widdowson 1960)

(all values as grams/100 grams foodstuff)

	Moisture	Nitrogen			Fat	Carbohydrate			SO_2	Thiamine HCl
		Total	Purine	Protein		Total	As starch	As		
Freshly manufactured pork sausage	50.7	1.41	-	8.8	28.8	9.8	-	-	0.45	0.0013
Lean Pork, freshly butchered	74.9	3.58	-	22.4	2.6	nil	-	-	nil	0.001
Sausage rusk (estimated)	5.0	1.50	-	8.8	1.2	89	89	-	nil	0.00025

TABLE 8

Component acids of Pig depot fats (Hilditch 1956)

(all values as grams/100 grams fat)

Carcase location of fat	Iodine Value	Saturated				Unsaturated				
		C_{12}	C_{14}	C_{16}	C_{18}	C_{14-1}	C_{16-1}	C_{18-1}	C_{18-2}	C_{20-22}
Perinephric	56.4	-	0.9	29.3	17.4	0.3	1.8	40.3	8.1	1.9
Inner back	58.9	0.1	0.8	27.5	15.1	0.2	1.7	44.2	7.8	3.1
Outer back	63.9	0.1	0.9	26.5	12.8	0.2	1.9	46.8	7.9	2.9

MATERIALS AND METHODS

Some microbiological experiments (1, 2A and 2B, Table 9), carried out in co-operation with Bath University, involved sausages incubated at 4°C and room temperature (ca. 20-22°C). All experimental work at T. Lucas (Bristol) involved sausages stored at 4°C for one day after manufacture, and subsequently at 20-22°C.

1. SAUSAGE COMPOSITION AND MANUFACTURE

All sausages were prepared to the basic formula (w/w): 65% total meat, 20% water, 12.5% sausage rusk, 2.5% sausage seasoning. All meat was derinded pork belly, cut into ca. 4" pieces. The sausage rusk was a standard Lucas grade ('DYR') of medium granularity (mean particle size 0.7mm ± S.D. 0.4mm). The seasoning used was formulated to one of the company's recipes ('Viscount Pork with Preservative and Polyphosphate'); in experiments in which SO_2 was not included (Table 10) the formula of the seasoning was changed to omit its precursor, sodium sulphite, and the percentage deficit made up with heat-treated flour. All water was added as ice.

A bowl-chopping production method was used. Meat, seasoning, water and half of the rusk, were comminuted first for 30 seconds. The machine was stopped, the remaining rusk added, and the mixture further

comminuted for ca. 60 seconds, to the desired texture. After extrusion, using a piston filler, into synthetic hog-sized casings (Devro Limited) and twisting into links, the sausages were hung to condition, at 4°C, for 24 hours, and then wrapped, eight to the pound, in thin-guage polythene, for subsequent storage.

2. EXPERIMENTAL FORMULATIONS

(a) Effect of SO_2

Sausages were prepared using seasoning without preservative, and SO_2 and alternative anti-microbials were incorporated, during the first stage of mixing, at the following levels per kilo of mix: sodium sulphite 6.5mM, sodium arsenite 6.5mM, sodium fluoride 10mM, and 2 : 4 : dinitrophenol 60mM (Tables 10 and 11).

(b) Effect of Alternatives to Sausage Rusk

Equal weights of breadcrumb, 70% extraction soft wheat flour, heat-treated 70% extraction soft wheat flour, and purified plant cellulose (Solkafloc, C.P.C.) were substituted for sausage rusk. In the cellulose sausage, flour present in the seasoning was similarly replaced (Table 12).

(c) Effect of Glucose

Sausages made with cellulose, and SO_2, were

continued page 62

TABLE 9

Experimental work on standard formula sausages, with/without SO_2

No.	Formulation	Type	Aim	Micro	pH	Acids	Fats	Qual. Organo-leptic	Quant. Organo-leptic
1	Rusk + SO_2	Co-op	Review micro	x	x			x	
2A	Rusk + SO_2	Co-op	Effect SO_2	x	x		x	x	x
2B	Rusk, no SO_2	Co-op	" "	x	x		x	x	x
3	Rusk + SO_2	TL	Organoleptic trials	x	x			x	x
4	Rusk + SO_2	TL	" " "	x	x			x	x
5	Rusk + SO_2	TL	Organoleptic trials (Graph rating)	x	x			x	x
6A	Rusk, no SO_2	TL	Production of acids	x	x	x		x	
6B	Rusk + SO_2	TL	" " "	x	x	x		x	

(Key - see over)

TABLE 9 (Continuation)

KEY

Co-op:	Co-operative experiments with Bath University
TL:	Experiments carried out at T. Lucas
Micro:	Microbiological tests - see Table 15
pH:	pH determination - see page 65
Acids:	Organic acids - see page 67
Fats:	Analysis of fats - see page 68 (et sequ)
Qual/Quant Organoleptic:	Taste panel checks - see page 70 (et sequ)

TABLE 10

Inclusion rates and postulated site of action of antimicrobials used in the thesis

Antimicrobial	Inclusion Rate per kilo sausage	Nature of Antimicrobial Action	Reference
Sodium sulphite	6.5mM	Yet to be identified	-
Sodium arsenite	6.5mM	Inhibition of respiration for α-oxoglutarate and pyruvate by blocking dilydrolipoyl dehydrogenase	Massey & Veeger 1961
Sodium fluoride	10mM	Formation of fluorophosphates: inhibits phosphoglucomutase and enolase activity. Effect inhibitor of glucose catabolism	Mahler & Cordes 1971
2 : 4 : dinitrophenol	60mM	Uncouples oxidative phosphorylation	Mahler & Cordes 1971

TABLE 11

Experimental formulations evaluated to assess effect of SO_2

(See also Table 10)

No.	Formulation	Type	Aim	Micro	pH	Acids	Fats	Qual. Organ.	Quant. Organ.
7A	Rusk	Solo	Compare and	x	x			x	
7B	Rusk + SO_2 (C)	"	contrast alternative	x	x			x	
7C	Rusk + ASO_2	"	antimicrobials to	x	x			x	
7D	Rusk + F	"	SO_2 in terms of	x	x			x	
7E	Rusk + DNP	"	effect on microflora,	x	x			x	
7F	Rusk + F + SO_2	"	pH and qualitative	x	x			x	
7G	Rusk + F + ASO_2	"	organoleptic features	x	x			x	
7H	Rusk + F + DNP	"		x	x			x	

X : experimental work carried out

TABLE 12

Experimental formulations containing alternative carbohydrates to sausage rusk

No.	Formulation	Type	Aim	Micro	pH	Acids	Fats	Qual. Organoleptic	Quant. Organoleptic
8A	Rusk + SO_2 (C)	Solo	To compare	x	x			x	
8B	Crumb + SO_2	"	and contrast	x	x			x	
8C	Flour & SO_2	"	the effects of	x	x			x	
8D	H.T.Flour & SO_2	"	alternatives to	x	x			x	
8E	Cellulose & SO_2	"	sausage rusk	x	x			x	

(C) : Control

All substitutions were on an equal weight basis

TABLE 13

Experimental formulations to assess the effect of the availability of glucose

No.	Formulation	Type	Aim	Micro	pH	Acids	Fats	Qual. Organoleptic	Quant. Organoleptic
9A	Rusk + SO_2	Solo	To compare and contrast the effect of availability of glucose to the developing association	x	x			x	
9B	Cellulose + SO_2	"		x	x			x	
9C	Cellulose + SO_2 + 0.001% glucose	"		x	x			x	
9D	Cellulose + SO_2 + 0.01% glucose	"		x	x			x	
9E	Cellulose + SO_2 + 0.1% glucose	"		x	x			x	
9F	Cellulose + SO_2 + 1.0% glucose	"		x	x			x	

TABLE 14

Experimental formulations evaluated to determine whether thiamine stimulates microbial growth

No.	Formulation	Type	Aim	Micro	pH	Acids	Fats	Qual. Organoleptic	Quant. Organoleptic
10A	Rusk + SO_2	Solo	To assess the effect of thiamine HCl on the organoleptic stability and microbiology of sausages	x	x			x	
10B	Rusk + SO_2 + thiamine HCl	"		x	x			x	
10C	" "	"		x	x			x	
10D	" "	"		x	x			x	
10E	" "	"		x	x			x	
10F	" "	"		x	x			x	
10G	" "	"		x	x			x	

Inclusion rate thiamine HCl (per kilo sausage meat)

Exp. batch no.	10B	10C	10D	10E	10F	10G
Thiamine HCl (mg)	0.63×10^{-4}	0.63×10^{-3}	0.63×10^{-2}	0.63×10^{-1}	0.63	450

supplemented with D-glucose in decade intervals of concentration from 0.001 to 1.0% of the total sausage weight (Table 13).

(d) Effect of Thiamine HCl

Sausages of normal composition were supplemented with thiamine HCl in decade intervals from 0.63×10^{-4} to 0.63mgs/kilo of sausage, and in addition, one batch was prepared with 0.447g/kilo (Table 14).

3. GENERAL METHODS OF SAMPLING

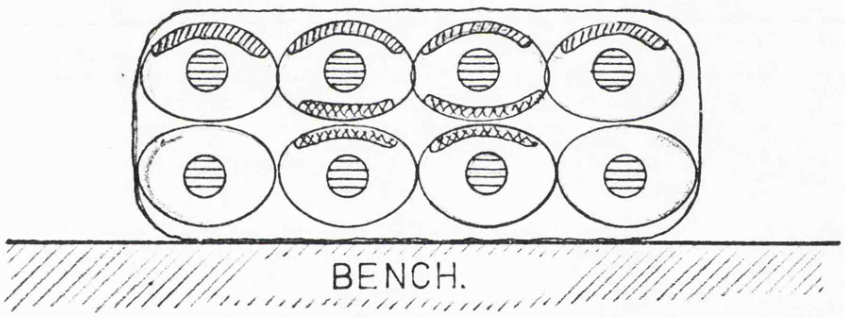
Four sites within the sausage were identified, for sampling purposes; the outer surface (O.S.), the inner surface (I.S.), a general surface (S) and a general core (C) (Fig. 6).

Sausages studied in co-operation with Bath University were sampled at the four sites, using the technique illustrated in Fig. 7. The core sample was recovered from the cork borer (No. 4) by displacement with a sterile glass rod.

Sausages studied at Lucas were sampled using the technique illustrated in Fig. 8. Only the outer surface, and core, sites were sampled. Surface incisions were to a depth of ca. 2mm. The core channel removed was ca. 1cm wide, 0.5cm deep, and terminated ca. 2cm from the ends of the sausage.

Fig 6

Diagramatic representation of a pack of sausages, in transverse section, showing the different sample locations.



Key:

S — General surface.

OS — Outside surface.

IS — Inside surface

C — Core.

Fig 7

Diagramatic representation of sampling methods used in co-operative studies.

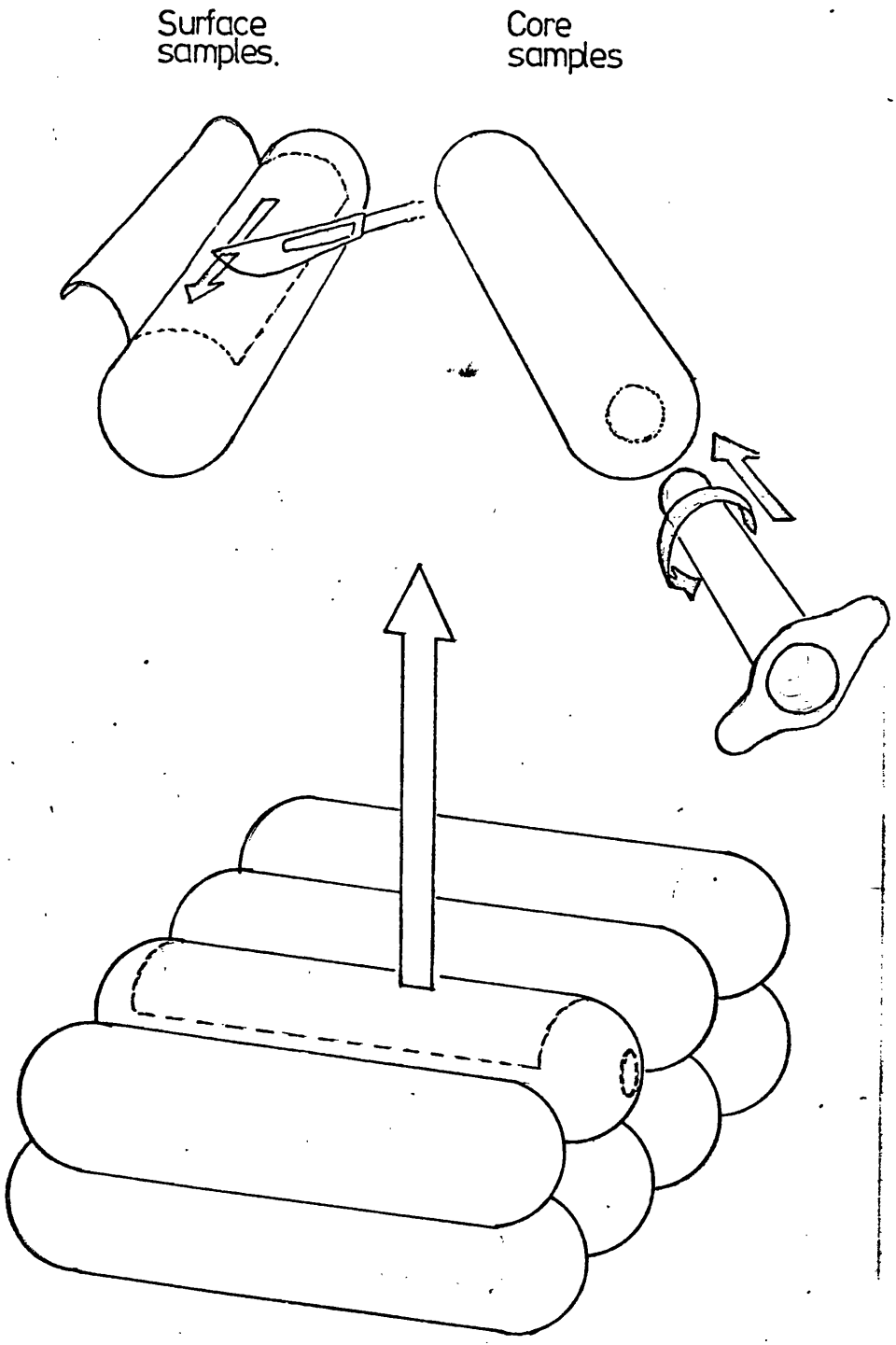
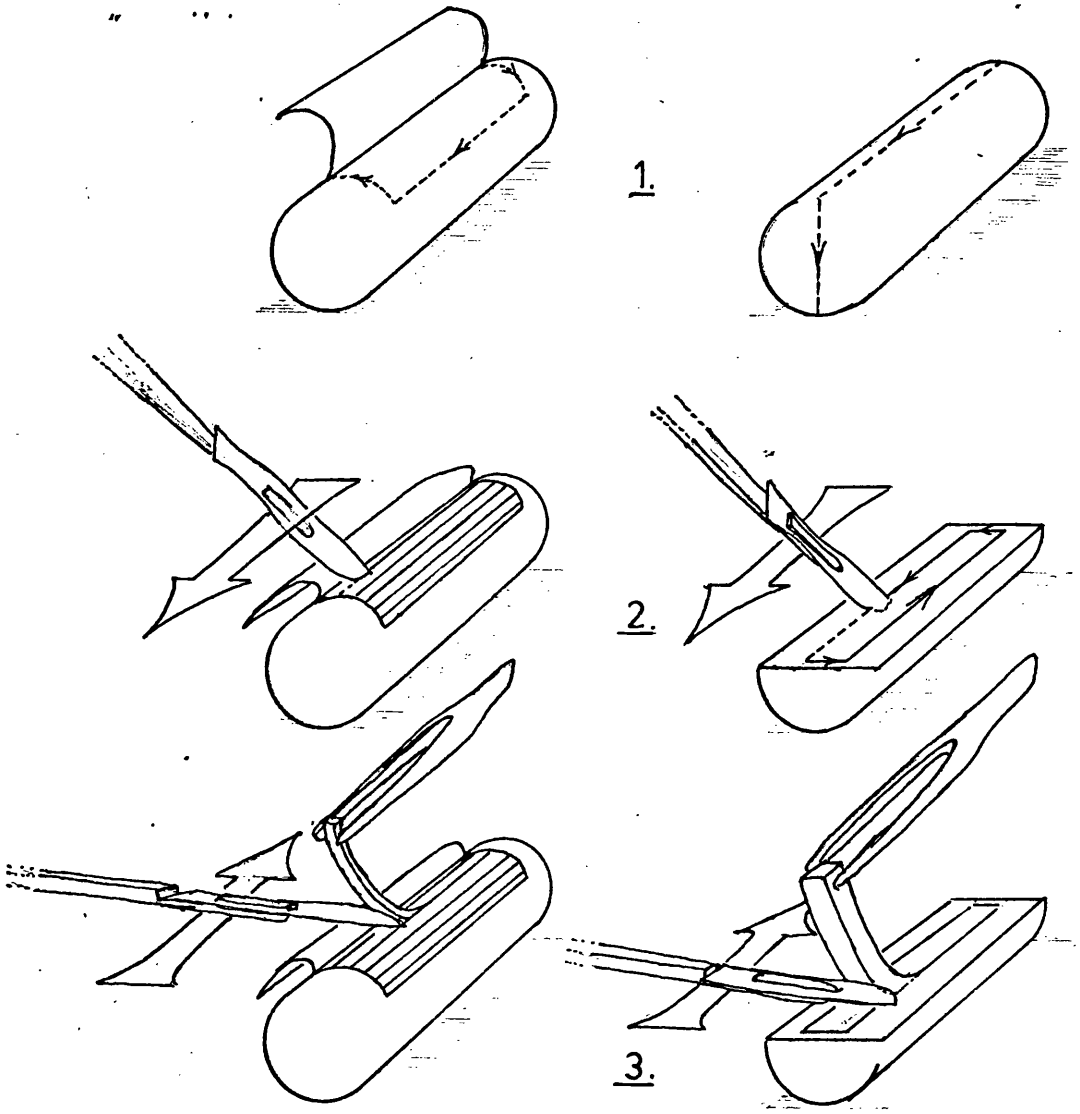


Fig 8
Diagramatic representation of the modified sampling procedure adopted at Lucas.



4. MICROBIOLOGICAL METHODS

10g samples of aseptically harvested sausage meat were macerated with 90cm^3 of sterile R/4 Ringer's solution for 1 minute at full speed in a top-drive homogeniser (M.S.E. Limited). Serial decade dilutions were made from this 10^{-1} suspension, in sterile R/4 Ringer's. The methods of enumeration were as given in Table 15, with the exception of batches 2A and 2B (Table 9) where a Colworth stomacher and dispenser/diluter were used, in accordance with the manufacturer's instructions (except for Keddie's medium, which was not suited because of its susceptibility to dessication).

After enumeration, the incubated plates were examined. Examples of the various colony morphologies on the two highest dilutions were picked off, and Gram stained preparations examined under the microscope.

5. BIOCHEMICAL METHODS

10^{-1} dispersions of sausage meat in distilled water were prepared using the method previously described.

(a) Measurement of pH

pH was determined on 10^{-1} aqueous dispersions using a pH meter (Pye Limited) fitted with a

TABLE 15

	Method of enumeration	Medium used	Temp. incubation	Period of incubation		Reference
				1st examination	2nd examination	
TVC	Pour plate	SPC agar	22°C	3 days	4 days	
M. thermosphactum	Pour plate	STAA	22°C	3 days	4 days	Gardner 1966
Psudomonas spp.	Spread plate	Masunosky	22°C	4 days	5 days	Masurvosky 1963
Yeasts	Pour plate	PCA-citrate	30°C	4 days	5 days	Dowdell & Board 1968
Lactobacillus spp.	Pour plate	Keddie	30°C	5 days	7 days	Keddie 1968
Coliforms	Pour plate	VRBA	37°C	1 day	-	

micro-spear combination electrode (Activion Limited) and automatic temperature correction. Sausage fat was removed from the electrode with a neutral detergent solution (2% v/v DECON-90), followed by rinsing with distilled water, after each determination. Calibration was performed using standard buffers (B.D.H. Limited) for pH 4 and 7.

(b) Determination of Organic Acids

The extraction, clean-up, and gas chromatography of organic acids (as their trimethylsilyl derivatives) in the filtrate of aqueous 10^{-1} homogenates, was performed using the method of Mamer and Gibbs (1973). 0.5µl aliquots were injected 'on-column' into a PYE 104 fitted with 3ft x ¼" O.D. glass columns packed with 3% (w/w) OV 17 (Phase-sep Limited), on Chromosorb W-AW, and chromatographed, using a nitrogen flow rate of 25cm^3/min., using the temperature programme: 2 minutes at 45^{o}C, 45-170^{o}C at 4^{o}C/min., 170^{o}C for 10 mins., cool. Detector conditions were optimised for the separation. Injector temperatures, set at maximum, were slaved to the oven temperature and maintained a constant differential of +100^{o}C. Retention times and elution temperatures

of standard acids were determined by calibration with pure derivatives.

(c) Changes in the Composition of the Fat

10g samples of sausage meat were homogenised (p.65) with 40cm^3 of distilled water, and centrifuged at 4^{o}C for 20 minutes at 12,000 r.p.m. (M.S.E. 18 - Fisons Limited). The chilled supernatant fat phase was recovered, and excess water removed from it on a No. 1 filter paper (Whatman).

1g samples of fat were extracted using the method of Bligh and Dyer (1959). The average recovery was 90% (w/w); the difference was attributed to water and connective tissue.

10µl aliquots of the chloroform extracts were chromatographed on thin layers of Silica gel G (Schleicher and Schull Limited) of dimensions 20cm x 5cm x 100µm, using the method of Bandyopadhyay and Gholap (1973). After evaporation of the solvent in a stream of warm air (2 hours), the spots were located by charring with methanol : sulphuric acid (9 : 1) at 110^{o}C for 10 minutes. Photocopies of the plates (Xerox Limited) were taken, their spots cut out

and their proportions estimated gravimetrically.

(d) Methylation of Fatty Acids

50µl aliquots of the chloroform phase were chromatographed on plates 1000µm thick (p.68). The location of the free fatty acid, and glyceride, regions of each plate were estimated by comparison with standard plates, and the individual fractions scraped off into foil dishes. Their component fatty acids were esterified by methylation with boron trifluoride-methanol (van Wijngaarden 1967). 1µl aliquots were chromatographed isothermally in a Perkin-Elmer F11 chromatograph equipped with 6ft. x ¼" O.D. stainless steel columns packed with diethylene glycol succinate at 20% on Chromosorb W-AWDMCS (Perkin Elmer FFA packing, standard column), at a nitrogen carrier gas flow rate of $5cm^3$/min. Detection conditions were optimised for the chromatogram, in the ionization amplifier range 2×10^2 to 5×10^2. Injector block temperature settings were scale value 6. Retention times were calculated by chromatography of standard mixtures (Sigma Biochemicals Limited), and peak areas used to calculate molar response coefficients.

6. SENSORY EVALUATION METHODS

All sensory evaluations were carried out by volunteer panellists.

(a) Appearance

Packs of sausages were placed in a refrigerated open-topped display cabinet, against a white background, and illuminated with flourescent light tubes with a colour temperature equivalent to northern hemisphere mid-summer daylight (Philips Limited).

(b) Aroma

Portions of half a sausage, taken from the exterior of the pack, with their outer surface uppermost, were placed in wine glasses, the mouths of which were subsequently covered with 3" diameter watch glasses. After equilibration of the aroma in the head space (ca. 5 mins) the contents of the glass were smelled once, and the watch glass quickly replaced.

All assessments were conducted in booths, under sodium lighting, at room temperature.

(c) Flavour

Sausages were baked in a pre-heated oven at 350^{0}F, for 40 minutes and turned 90^{o} about their longtitudinal axis every five minutes

for the first 20 minutes. Portions of half a sausage were presented on paper plates for flavour assessment, in booths, under sodium lighting.

(d) Method of Presentation - quantitative trials only

Samples for sensory evaluation using the category scale (Fig. 9) or graph-rating scale (Fig. 10) were accumulated in a deep freeze (-30°C) over a period of one week, thawed, coded, randomised and presented singly for assessment on a 'double-blind' basis (i.e. their identity was unknown to either the presenter or the assessor).

(e) Description of Stimuli

Panellists were asked to give a written description of their opinions during qualitative assessments. A standard vocabulary was not used.

(f) Quantification of Stimuli

Responses to stimuli were quantified using the scales previously mentioned (Figs. 9 and 10). Use of the hedonic scale required agreement, signified by a tick, with one of the descriptions offered. With the graph-rating scale, a pencil line at 90° to the scale was required to indicate the assessed position of the response in relation to the limits specified. This was subsequently translated into a numerical score by measuring the distance of the pencil line (in mm) from the left hand end of the scale.

Fig9 The hedonic scale used for category-ratings.

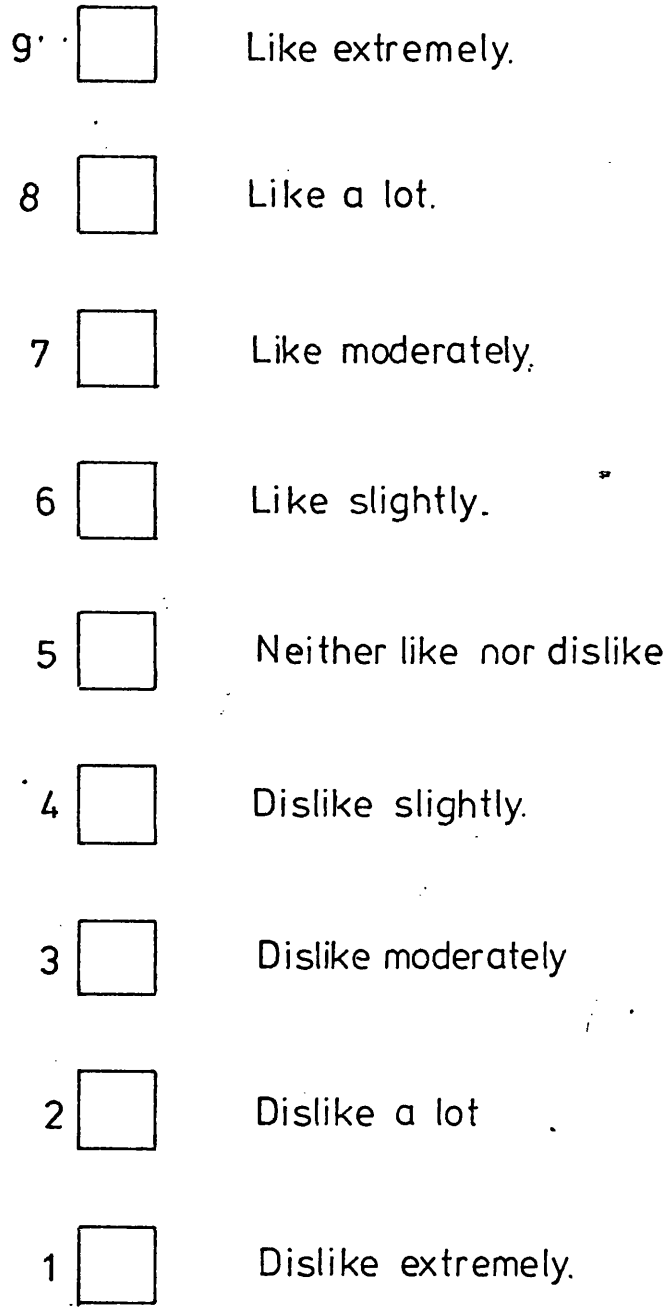
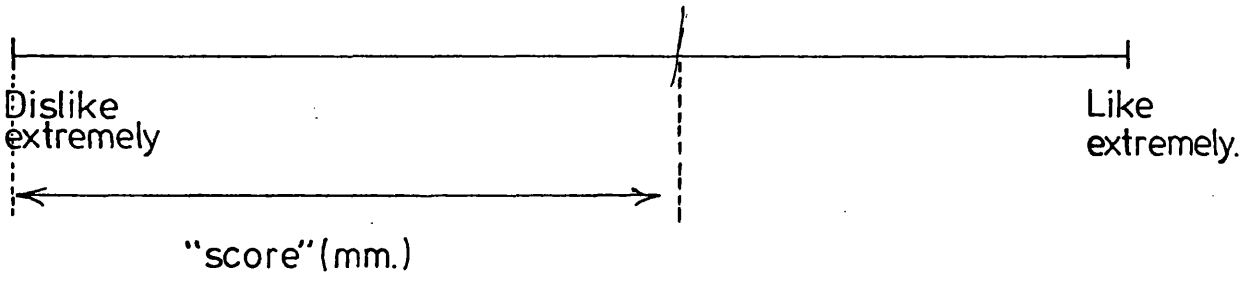


Fig10 The scale used in the graph-rating experiment.



RESULTS

Qualitative Changes in the Organoleptic Characteristics of Pork Sausages

The spoilage of sausages is characterised by the development of unacceptable organoleptic qualities, traditionally viewed as caused by microbial growth. Rusk, the most common cereal used in sausages, has evolved as an alternative to breadcrumbs by virtue of its superior colour, water absorption, and yeast-less manufacturing process (aeration being achieved and controlled using ammonium bicarbonate). Abbiss (1978) has demonstrated transient porcine and yeast amylases in pork sausages, and sausage rusk starch is regarded as their principal substrate, and a major source of energy for microbial growth.

Rusk is gradually becoming more expensive as a raw material for sausages, and many manufacturers are using or considering alternatives (e.g. flours, texturised soya proteins) to maintain profitability. However, the evolution of rusk as an ingredient suitable for sausages might have been influenced by considerations other than those of colour etc. mentioned above (e.g. organoleptic characteristics). This possibility was investigated by comparing a range of carbohydrate polymers in sausages, and also by assessing the effects of alternative antimicrobials, and high initial glucose concentrations.

Changes detected in the aroma

Similarity matrices for the aromas of the range of experimental sausage formulations were constructed, to include both fresh (Fig. 11) and spoiled products (Fig. 12). A matrix comparing the aroma qualities of spoiled sausages from all the trials, in terms of three main aroma types identified, has also been drawn (Fig. 13). The following effects were observed:

(i) The effect of different carbohydrate sources

A relationship existed between the type of carbohydrate included in the formulation, and the initial aroma of the fresh sausage. For the standard pork sausage the aroma seemed to combine a smell of 'baked cereal' from the rusk, the aroma of pork fat, and those of the volatile components of the spices. The substitution of breadcrumb for breadrusk did not alter the aroma character, probably because a baking stage is involved in breadcrumb production, and hence the 'baked cereal' element was present. Raw flour and heat-treated flour, did not contribute this aroma component. Sausages containing these ingredients had initially an aroma which slightly resembled the control (rusk) due to the presence of a cereal, rather than baked cereal, smell of the flour, but it was weaker. The odourless

cellulose did not contribute to the aroma, and sausages containing it smelled only of spiced pork fat.

The baked cereal aroma component seemed to complement the spices, amplifying their contribution to the sausage's character in a synergistic manner.

A second relationship linked the amount of processing the cereal had received (Fig.12) to the intensity of the final spoilage aroma. Spoiled sausages made from flour had the most intense smell (described as 'yeast-like', and 'fermented', and described in the next section), and the rate of its development was the most rapid for sausages containing preservative. Heat-treated flour, breadcrumb and sausage rusk produced spoilage odours of the same general character to that of flour, but of lesser intensity. The spoiled aroma of cellulose sausages was similar in character, but much weaker, and it developed more slowly. Supplementation of cellulose with 1% w/w α : D : glucose produced (after ca. 4 days at 22°C) an aroma similar in intensity to flour, and its rate of development was comparable.

(ii) The effect of alternative antimicrobials

With the exception of 2 : 4 : dinitrophenol, which

gave a strong disinfectant smell, the inclusion of alternative antimicrobials to sulphur dioxide did not result in a markedly different aroma in the fresh product. The spoiled aromas differed considerably, and have been grouped thus:

(a) 'Cheese-like'

This highly objectionable aroma had the qualities of strong, over-ripe cheese. Its rate of development was such that sausages exhibiting it were rejected by an untrained panel within 2 days of their manufacture. It was the characteristic spoilage aroma of sausages in which sulphur dioxide had been omitted. It developed also in sausages containing sodium fluoride.

(b) 'Sweet and sickly'

This aroma combined a strong cheese-like quality with a sweet, perfume aroma and a faint smell of diacetyl. It was detected in sausages containing 2 : 4 : dinitrophenol, alone or in combination with fluoride. Although unpleasant, it did not become objectionable to the taste panel until 4-5 days of incubation at 22°C had passed.

(c) 'Yeast-like and fermented'

This aroma was the most complex and was typical of the spoilage of sausages containing rusk and

sulphur dioxide. It comprised several components; a yeast-like, alcoholic, warm, dry aroma, which was the most noticeable component; a faint, acid, sour smell, and a background aroma of cereal (rather than baked cereal) and rancid pork fat. Sausages containing arsenite, sulphur dioxide, and combinations of fluoride and arsenite, and fluoride and sulphite, all exhibited this type of aroma, at varying strengths. In the control sausage (rusk and sulphur dioxide) its intensity becomes objectionable between the fourth and fifth day of storage at 22°C.

(iii) The effect of herbs and spices

Immediately after manufacture, the volatile oils from herbs and spice ingredients were not easily detected in the fresh sausage's aroma. Their presence became more noticeable in the first day of storage, after which their contribution faded. After storage for three days at 22°C, it was difficult for the taste panel to detect them. Moreover, their detection in the presence of the spoilage aroma was difficult, and they therefore were (apparently) lost more rapidly from sausages which spoiled quickly (i.e. flour). It has not been demonstrated whether or not this is a true, or a masking, effect. Sausages prepared from cellulose did not smell as aromatic or 'spicy' as similarly formulated sausages

containing rusk. It is proposed that there is synergism between the aroma of breadrusk and the aromas of some spices, in particular nutmeg and mace.

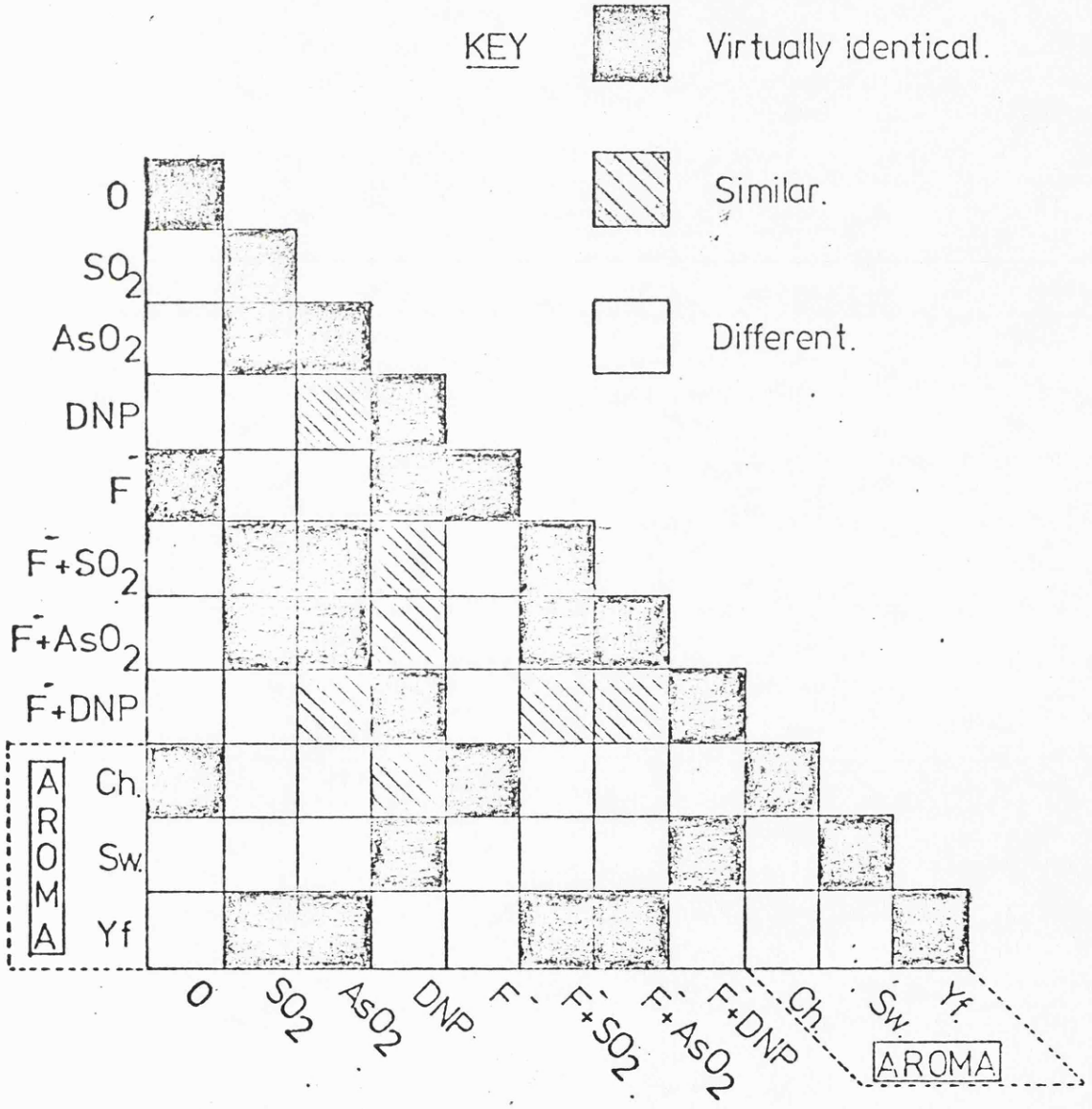
(iv) The effect of thiamine

The supplementation of sausages containing sausage rusk and sulphur dioxide with thiamine-HCl at various levels up to 450p/10^6 did not cause any change in the character or strength of the aroma during spoilage, compared with the control.

The results from this section suggested that the fresh and spoiled aromas of sausages were influenced by the type of carbohydrate used in their manufacture, the manner of its production, and its susceptibility to degradation.

Of the sensory criteria used by the public to assess sausages, colour and aroma are probably the most important to the initial impression. Aroma may be a more consistent guide because colour may be controlled using dyes, anti-oxidants and colour-holding additives. For a constant set of conditions (with regard to sausage composition) changes in colour might relate to carbohydrate in a similar manner.

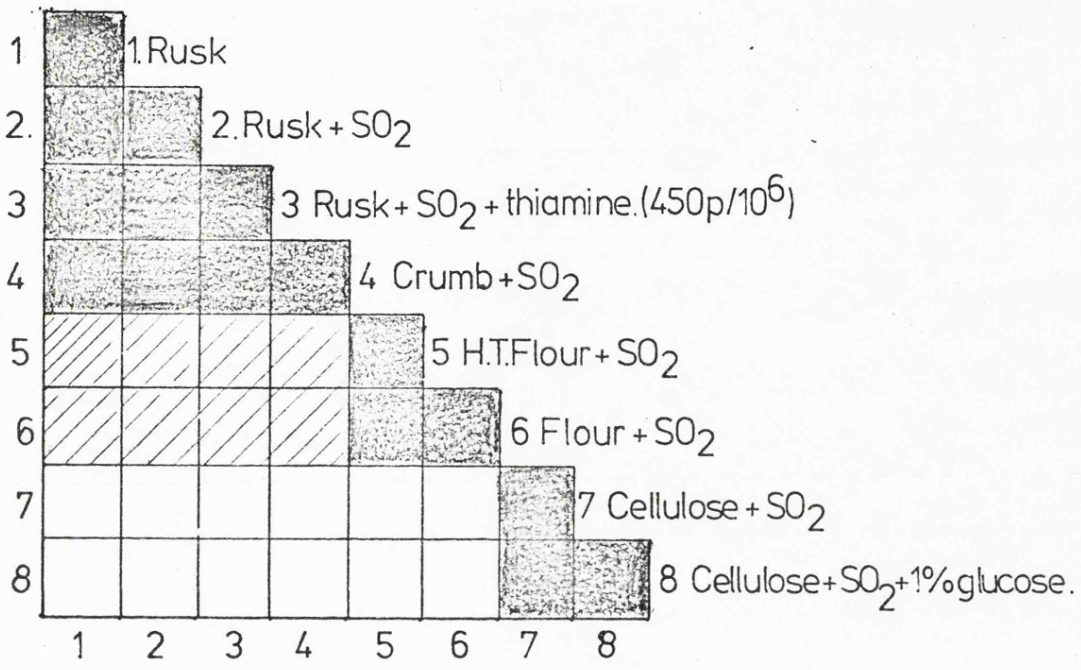
Fig.11 Odour-similarity matrix for Rusk-filled sausages containing various metabolic blocking agents.[4 days 22°C]



Aroma notation: (i) Ch.:- cheese-like. (ii) Sw.:- sickly-sweet & cheese-like.
(iii) Yf.:- yeast-like, fermented.

Fig 12 Odour-similarity matrix for carbohydrate polymers. (Key as for Fig 11).

AT START.



AFTER 5 DAYS at 22°C

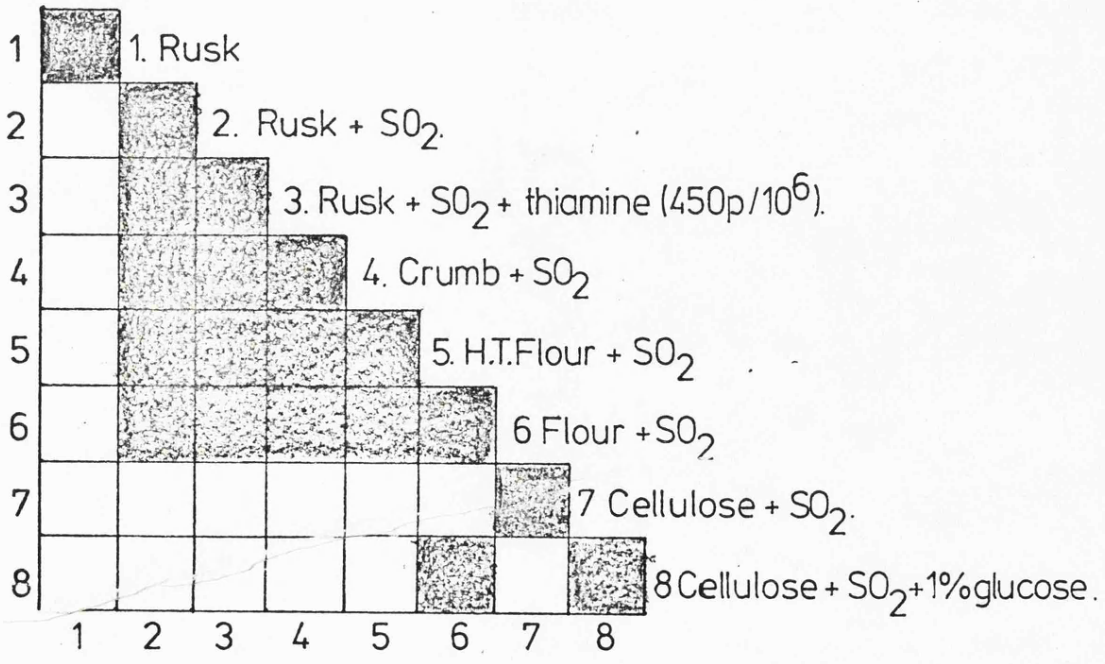


Fig13: Similarity matrix for odour similarity & classification: all trials.

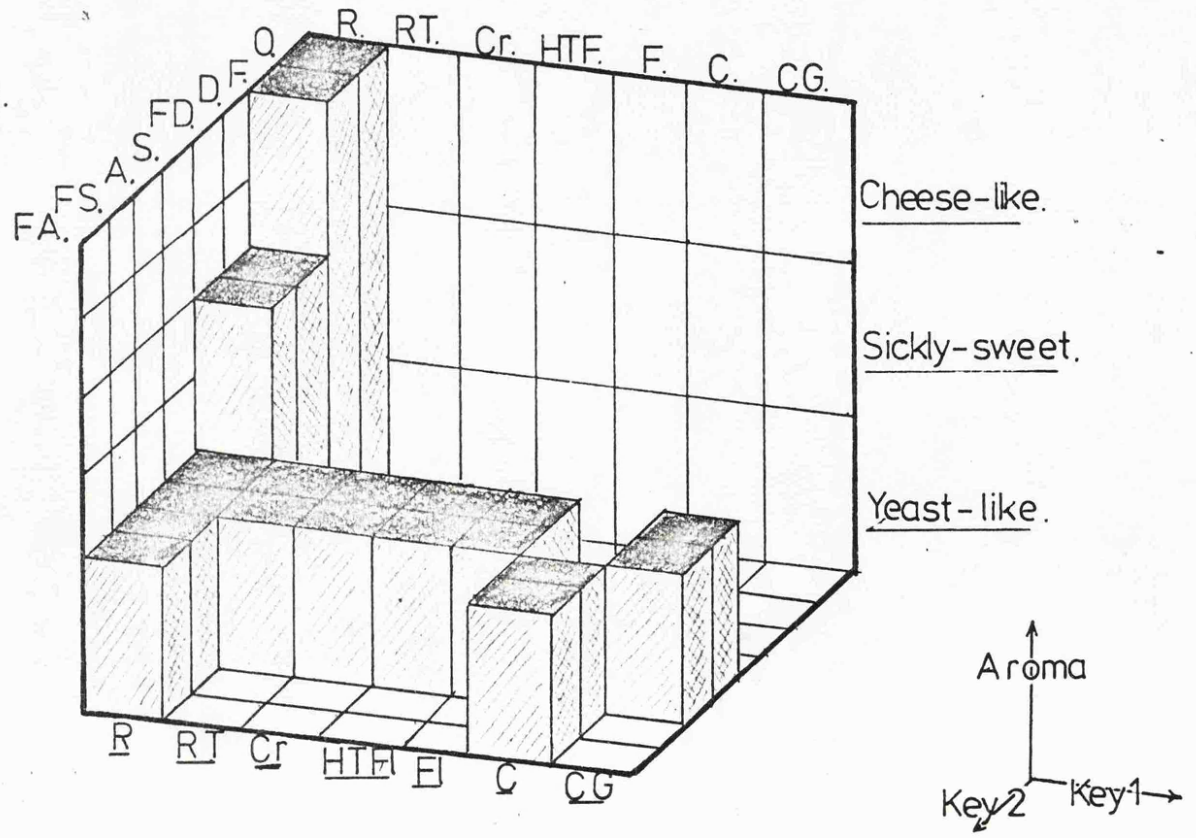
KEY

1. Carbohydrates.

R : Rusk.
RT: Rusk + thiamine HCl [450p/10^6].
Cr : Crumb.
HTF: Heat-treated Flour.
F : Flour.
C : Cellulose.
CG: Cellulose + 1% glucose.

2. Blocking agents.

F : F^-
D : 2:4:D.N.P.
FD: F^- + 2:4:D.N.P.
S : SO_2.
A : AsO_2^-.
FS : $F^- + SO_2$.
FA : $F^- + AsO_2^-$.



Changes observed in the colour

By controlling the texture of the mix, and standardising on a 2oz sausage, the number of variables contributing to the perception of change in the appearance of the product was reduced from five to three: colour (chromaticity),'shinyness' (reflectance) and greyness (hue). The changes, and influence of these on the acceptability of sausages was sought.

Changes in the colours of the sausages studied are illustrated in Fig.14 . They have been grouped into four categories of colour change, ranked in order of increasing loss, in Fig.15 . The following features are worthy of note.

(i) The effect of carbohydrate

The influence of the carbohydrate polymers used at 12.5% w/w in the experimental sausages can be ranked according to the amount and type of processing they had received during manufacture (Fig.15). Effects related to this are (a) the more refined the carbohydrate (the order being cellulose > rusk, breadcrumb > heat-treated flour > flour) the less the change in the colour of the sausage during storage at 22°C, and (b) treatment of the carbohydrate with heat during manufacture conferred some stability against colour loss in the sausage.

The particle size of the carbohydrate polymers also affected the colour of the product. The very fine

particle size of the flours and cellulose gave a very reflective background to the mixture, making them appear brighter. Flour imparted an off-white, and cellulose a white, colour; the latter therefore makes the sausage appear cleaner and less grey. Their fine particle size also obliterates the individual meat particles in the matrix, creating an impression of a very fine, smooth texture (Plate 2).

Comparatively, the rates of colour change in sausages with respect to carbohydrate present can be ranked in the descending order: flour/cellulose + 1% glucose > heat-treated flour/rusk/breadcrumb > cellulose (Fig.15). It is interesting to note that supplementing cellulose with 1% (w/w) α: D : glucose increased the rate of colour loss. Overall therefore there would seem to be a relationship between the availability of fermentable carbohydrate, and colour loss, in which the manufacturing process for the carbohydrate plays a part. An alternative means of investigating this hypothesis is to selectively block the availability of carbohydrate, and alternative energy-sources, and note the effects.

(ii) <u>The effect of alternative antimicrobials</u>

The colour changes occurring in sausages containing

various combinations of alternative antimicrobials (p.57) are illustrated in Fig.14 and categorised in Fig.15 . The effects of 2 : 4 : dinitrophenol are not included because of its bright yellow colour.

Overall, colour loss in sausages containing rusk was most effectively reduced by combinations of either fluoride and arsenite, or fluoride and sulphite. These pairings gave better results than arsenite or sulphite alone, which in turn gave significantly better protection than fluoride. The observed colour change for the latter was the same as for sausages containing rusk, but no sulphite. The strongest effect therefore seems to be exerted by arsenite or sulphite, and in their presence fluoride exerts a demonstrable effect. In their absence, the effect of fluoride is neutralised, and the sausage colour decays at the higher rate previously described (p.83). From the results it was apparent that sulphur dioxide has a strong colour holding effect, which can be marginally improved by incorporating fluoride. However, the effect of sulphur dioxide (and to an equal degree arsenite) seems to be crucial to the maintenance of the sausage's initial colour. As fluoride was incorporated to restrict the catabolism of carbohydrate, which is assumed (Abbiss 1978) to be the principal energy source in the sausage, the

specific colour holding effect of sulphur dioxide might not be directly linked with carbohydrate metabolism.

(iii) The effect of micro-organisms

Although the traditional view has been that spoilage of sausages, and deterioration of their colour, is caused by the effects of the growth of micro-organisms, it was noted during this study that there may also be a physical effect caused by microbial growth. Small colonies of micro-organisms, morphologically similar to Micrococcus spp. (when observed under the microscope) were observed to grow on the exterior surface of the sausage casing. In the presence of sulphur dioxide their growth appeared to be dependent upon the carbohydrate used. Thus the best growth was found with flour, the least with cellulose, and with the remaining fillers intermediate. The organisms did not appear on sausages containing fluoride and sulphite, fluoride and arsenite, and 2 : 4 : dinitrophenol, but could be observed on sausages containing sulphite, and arsenite. They were plentiful on sausages made from flour, and from sausages made with rusk without preservative. In the last two cases their growth eventually coalesced to form a thin paste-like layer. Colour change under these conditions might therefore be a matter of the slow physical obliteration of the sausage meat mixture by the developing microbial film. The colony colour of these

organisms after growth on Plate Count Agar (Lab-M Limited) at 22°C is similar to that of spoiled sausages.

The loss of colour in sausages would therefore seem also to be dependent upon the type of cereal used as an ingredient, its ability to contribute carbohydrate to the sausage, and the growth of micro-organisms.

Plate 2

The effect of the substitution of flour for rusk on colour and reflectance.

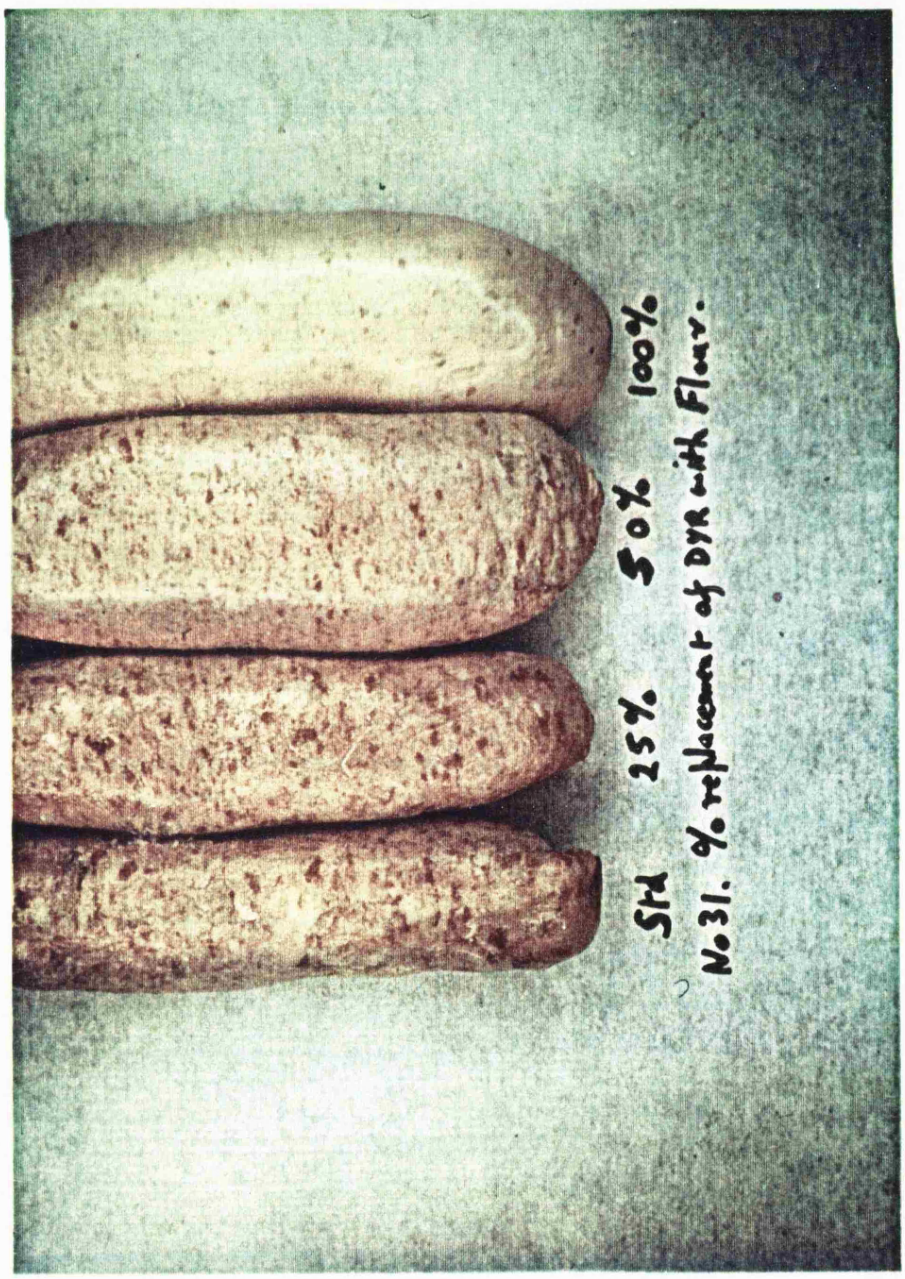


Fig 14 Changes in surface colours over 5 days at 22° C.

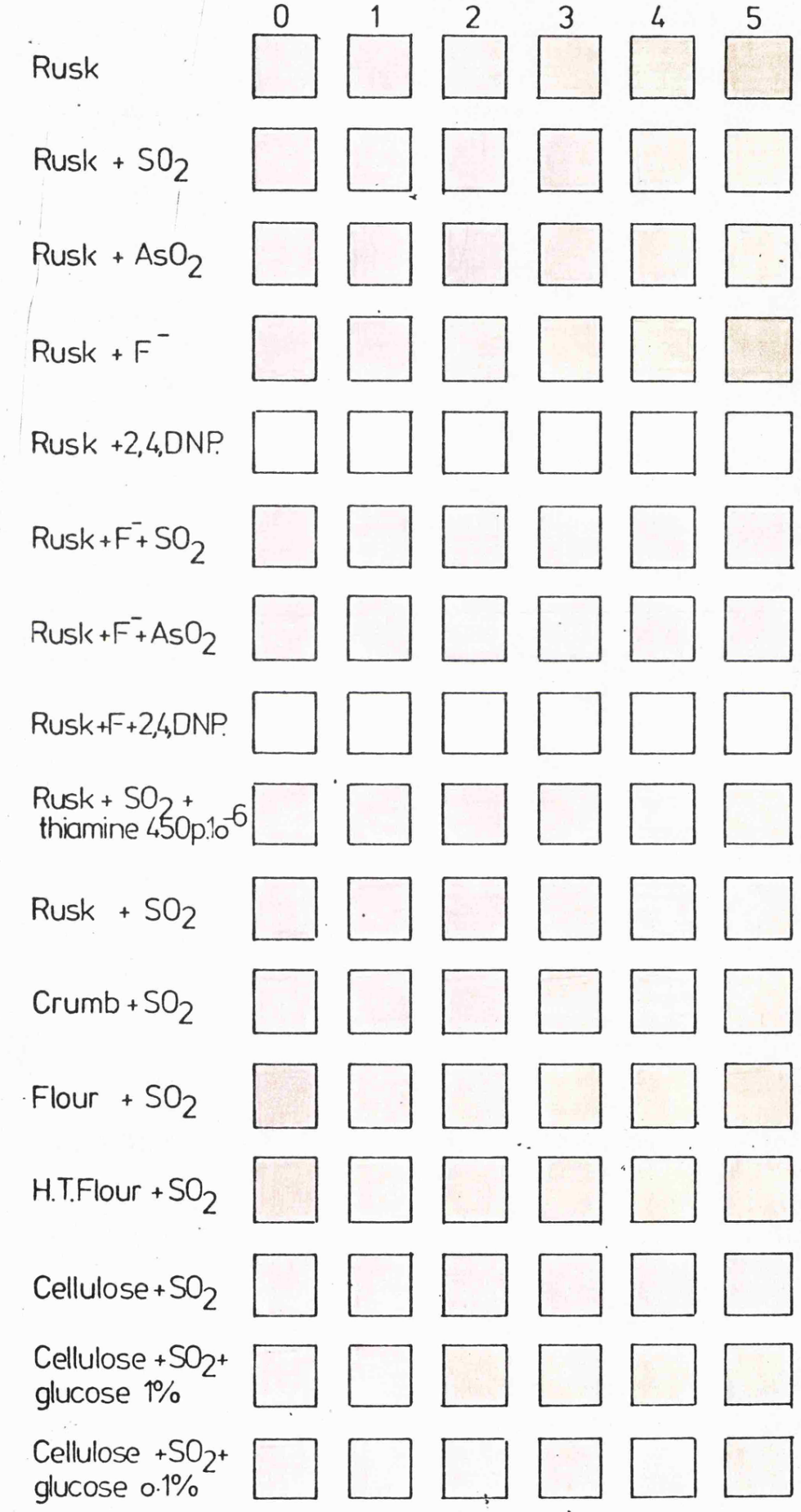
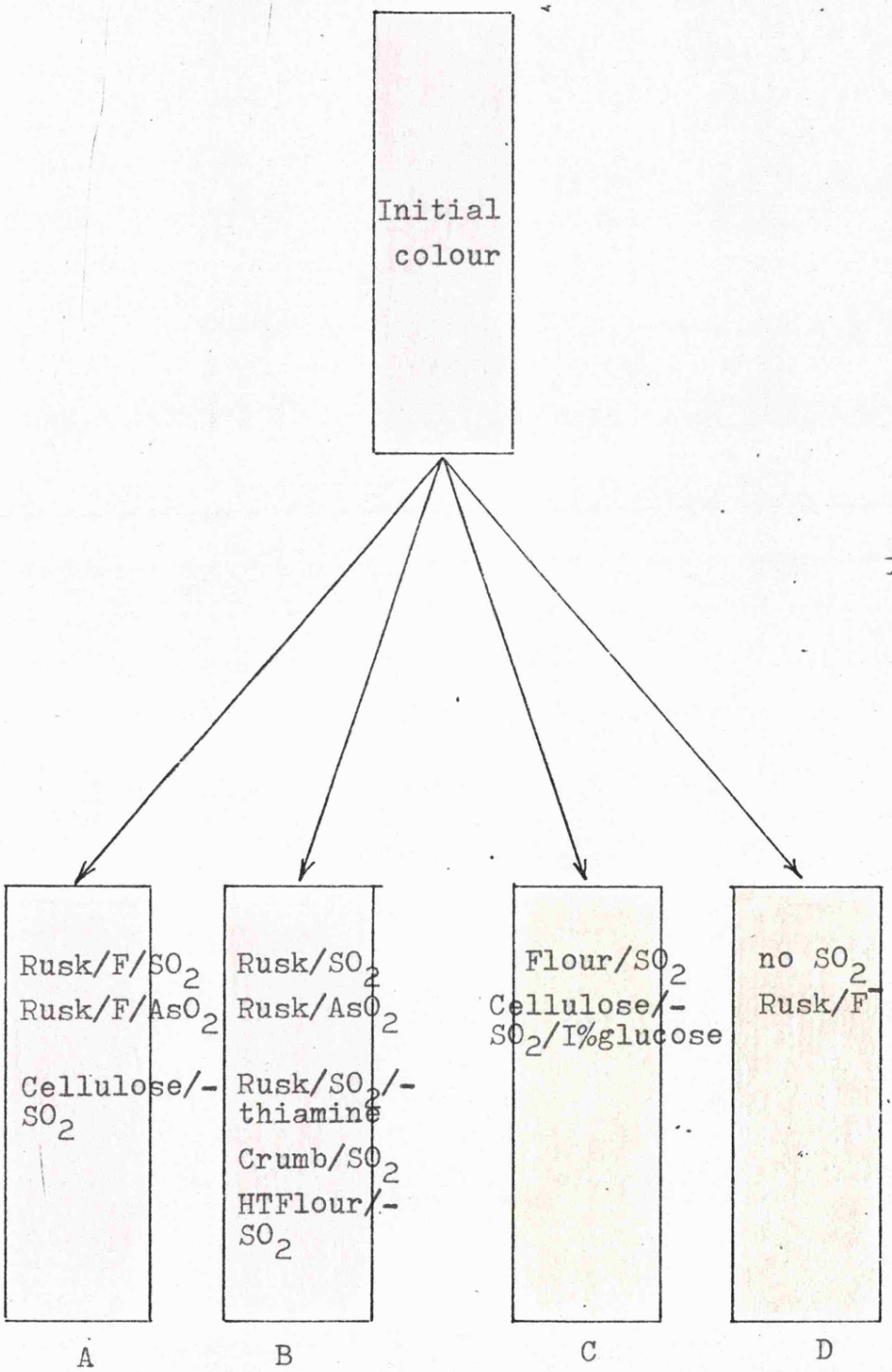


Fig 15 The four categories of colour change [Fig 14]



Changes detected in the flavour

The results in this section are limited to observations of sausages made from rusk, flour, heat-treated flour or breadcrumb, and containing sulphur dioxide at 450p/10^6. A comparative assessment was also briefly made using sausages containing rusk but without preservative, but the flavour of these quickly became so objectionable that the trial had to be terminated in order not to bias the panel against further trials of any kind.

Because they were poisonous, sausages containing fluoride, arsenite or 2 : 4 : dinitrophenol were not evaluated. The textural differences of cellulose sausages also made evaluation of their flavour difficult, because of their unusual mouthfeel.

The comparative trial for flavour of cooked sausages showed that three factors predominated:

(i) The inclusion of sulphur dioxide

Sausages containing sulphur dioxide at 450p/10^6 at manufacture, remained palatable at room temperature for up to 4 days. Ignoring the aroma, spoilage was subsequently detectable as a sour/acid taste. Even cooked, spoiled, preserved sausages, however, do not seem to be completely unpalatable. In contrast, sausages which did not contain preservative quickly developed a strong acid taste and were inedible after 2 days storage at room temperature.

Some assessors claimed that sulphur dioxide contributed to the aroma of fresh sausages; they were given a pungent, sharp, citrus quality which was not present in the unsulphited product.

(ii) The source of carbohydrate (for sausages containing SO_2)

The cooked flavours of sausages containing rusk and breadcrumb were regarded as very similar, both immediately after manufacture and when spoiled. The spoiled flavour was sour and acidic, with a slight cream-like after taste. Spoiled sausages containing flour and heat-treated flour were similar in character, but their unspoiled, initial taste seemed more bland. The development of the spoiled flavour was more rapid, and the flavour intensity greater, in the former. The flavour components from the baking of rusk and crumb were noted as contributing to the characteristic flavour of the sausage, a similar finding to that observed for their contribution to the aroma. It was noted that sausages which were just noticeably different in terms of flavour, and rejectable, were considered acceptable after cooking by some of the panellists. Although the subjective nature of the tests cannot eliminate this natural variation, two conclusions are possible: either the discrimination of the test was not sufficiently good to accomodate this kind of obser-

vation, leading to a consistent error in testing, or certain components of the spoiled aroma are very volatile or reactive, and are being lost or chemically changed during cooking. The cooking process chosen (p. 70) was designed to simulate as closely as possible the conditions likely to be used by the general public.

(iii) Volatile and non-volatile spice oils and resins

Unlike the phenomena noted for the aroma, added spice oils and oleoresins were detected immediately after manufacture, but their individual strengths decreased during storage. After three days at 22°C, the flavours of nutmeg and mace in the seasoning had disappeared from the sausage, regardless of whether flour, heat-treated flour, breadcrumb or rusk were used as a carbohydrate source, and sulphur dioxide was present. In contrast, the non-volatile components (e.g. piperine from peppers) did not fade in intensity.

Overall, therefore, there seems to be a strong relationship between the type of carbohydrate included in the sausage recipe, the amount of processing it has been subject to during manufacture, its ability to contribute carbohydrate, and the stability of the organoleptic features measured qualitatively in this section. How these organoleptic characteristics change with time is reported in the next section.

QUANTITATIVE CHANGES IN ORGANOLEPTIC QUALITIES

The previous section dealt with overall qualitative changes perceived in the appearance, aroma and taste of pork sausages under various experimental conditions. In addition to characterising the type of change produced it was considered necessary to investigate the rates of change occurring in these sensory features, in order to relate them to other dynamic features of the sausage; the development of the microbial association, and biochemical changes.

1. Hedonic scaling experiments

Three experiments were conducted. The results were analysed statistically in order to determine (a) if the results from the individual trials were related, or were mutually independent, and (b) how well the measured stimuli correlated.

The mean responses for each day, and their variances, were compared using Student's t, calculated:

$$t = \frac{(\bar{x}_1 - \bar{x}_2) - (\mu_1 - \mu_2)}{\sqrt{S_p^2 \left(1/n_1 + 1/n_2\right)}}$$

Equation 1

(Hayslett & Morray, 1973)

The assumption was made that the observed individual means were samples from parent populations with the same mean, permitting the null hypothesis:

$$\mu_1 - \mu_2 = \Delta_0 = 0$$

Hence, Equation 1 may be rewritten:

$$\frac{(\bar{x}_1 - \bar{x}_2) - 0}{\sqrt{S_p^2 \, (1/n_1 + 1/n_2)}} \ll t \text{ for } H_o: \mu_1 - \mu_2 = \Delta_o \quad \underline{\text{Equation II}}$$

and its specific alternative:

$$\frac{(\bar{x}_1 - \bar{x}_2) - 0}{\sqrt{S_p^2 \, (1/n_1 + 1/n_2)}} \gg t \text{ for } H_1: \mu_1 - \mu_2 \neq \Delta_o \quad \underline{\text{Equation III}}$$

The results (Tables 16-20) show that trials I and 3 were very similar (for all mean responses) and satisfied equation II. For trials 1 and 2, and 2 and 3, the hypothesis was less robust, and for the specific comparisons of the mean aroma scores on days 4 and 5, and the taste scores on day 5, the specific alternative of Equation III had a greater probability of being true.

Correlation coefficients were calculated for the three possible stimulus pairings in the individual experiments (Table 18). The estimates were all significant ($P = \geqslant 0.1$), even though the number of trials studied was comparatively small. For sausages stored at ca. 20°C the calculated values of r. for each stimulus pair were consistent up to and including the fourth day of storage, after which the consistency changed.

Pooled correlation coefficients were calculated (by Z-transformation) for each stimulus pair for each trial, for all pairs for each trial, and for all trials for each pair (Table 20). Appearance v. aroma, and

appearance v. taste, had coefficients of similar magnitude and sign for each trial. The correlation of aroma with taste was not as good but was still significant. The experimental errors in the transformed data were similar for trials 1 and 2; trial 3 was more accurate, probably because it contained a greater number of samples.

From the above results it was concluded that there was confidence in the assumption that the trials were similar, and that the phenomena studied were linked.

Inspection of the results showed that scores for the individual stimuli all changed quite sharply after the fourth day of incubation (at ca. 20°C). This observation was tested using variance analysis to calculate and compare Snedecor's F statistic. Two analyses were made for each stimulus in each trial; the first for all observed values for each stimulus, from manufacture to the termination of the experiment; the second for a sub-sample of these observations, from manufacture to day 4.

Each calculated value of F (F_c) was compared to the predicted value (F_p) and their difference calculated (Tables 21-30). In all cases, the estimates of F were more significant for the whole sample than for the sub-sample. Thus, the observation that there appeared to be a sudden loss of preference for sausages after

the fourth day of incubation at ca. 20°C was assumed to be correct. Trials 1 and 3 had a similar value for F, greater than that observed for trial 2.

The ratio between the calculated values for F for the samples and the sub-samples was used to estimate the change occurring in each of the stimuli measured; this in turn was interpreted as an index of sensitivity to each stimulus and therefore a measure of its suitability as a quality indicator. The identification of an indicator is one of the stated aims of the thesis. The largest perceived change, in terms of F, occurred in the aroma, indicating that it was the most sensitive of the three stimuli for this particular judgement.

The suitability of aroma, supported by either of the other two stimuli, as non-destructive tests for use by Quality Assurance departments, was explored using multivariate regression analysis. Partial regression equations were extracted to estimate each stimulus in terms of the other two (Table 31) and to calculate their correlation coefficients and standard errors.

The correlation coefficients for the stimuli in each partial regression equation were all very similar, lying between +0.8 and +0.89. The standard errors of the three expressions derived to describe the aroma scores in the three trials were larger than the errors for taste and appearance respectively, but because of the

continued page 116

TABLE 16

Mean scores, variances and number of assessments for hedonic category scaling experiments (temp. storage 22°C)

	Age	Trial 1			Trial 2			Trial 3		
Parameter	Days	$\bar{x}$	o^2	n	$\bar{x}$	o^2	n	$\bar{x}$	o^2	n
Appearance	1				6.30	1.12	10			
	2	6.72	8.18	11				6.58	0.77	24
	3	5.38	7.56	13	5.22	1.93	9	5.35	1.19	20
	4	5.45	2.27	11	5.22	2.96	9	5.57	1.56	21
	5	4.70	2.90	10	4.33	0.67	6	4.94	3.06	17
	6				4.57	1.61	7			

	Age	Trial 1			Trial 2			Trial 3		
Parameter	Days	$\bar{x}$	o^2	n	$\bar{x}$	o^2	n	$\bar{x}$	o^2	n
Aroma	1				6.70	2.25	10			
	2	6.45	1.47	11				6.33	1.54	24
	3	5.30	1.06	13	5.22	1.93	9	5.79	3.73	19
	4	3.36	1.88	11	5.11	2.62	9	3.24	1.99	21
	5	2.00	0.89	10	3.50	1.90	6	2.12	1.85	17
	6				3.57	3.96	7			

Table 16 (Cont/.)

TABLE 16 (Continuation)

	Age	Trial 1			Trial 2			Trial 3		
Parameter	Days	$\bar{x}$	o^2	n	$\bar{x}$	o^2	n	$\bar{x}$	o^2	n
	1				6.50	1.82	10			
	2	6.72	2.62	11				6.42	2.34	24
Taste	3	6.38	1.42	13	5.78	0.94	9	6.15	2.25	20
	4	5.09	2.29	11	4.67	1.74	9	4.81	5.24	21
	5	2.80	4.18	10	5.17	0.17	6	2.65	4.12	17
	6				4.29	2.56	7			

(Hayslett & Murray, 1974.)

TABLE 17

Calculated values for t, for different trial comparisons

	Age	Trial 1 v 2		Trial 1 v 3		Trial 2 v 3	
Parameter	Days	t	DF(v)	t	DF(v)	t	DF(v)
Appearance	2			+0.89	33		
	3	+0.43	20	+0.14	31	-0.56	27
	4	+0.66	18	-0.47	30	-1.40	28
	5	+1.21	14	-0.64	25	-1.5.	21
Aroma	2			+0.49	33		
	3	+0.18	20	-1.15	31	-1.41	27
	4	-3.53	18	+0.41	30	+6.62	28
	5	-2.80	14	-0.37	25	+4.62	21
Taste	2			+1.03	33		
	3	+1.93	20	-0.71	31	-1.04	27
	4	+1.04	18	+0.62	30	-0.31	28
	5	-15.07	14	-0.37	25	+5.19	21

(Hayslett & Murray, I974.)

TABLE 18

Correlation coefficients for the stimulus pairs Appearance : Aroma (C : A), Appearance : Taste (C : T) and Aroma : Taste (A : T) for the category rating experiment

(a) Trial 1

Pair	Day 2	Day 3	Day 4	Day 5
C : A	+0.88	+0.85	+0.91	+0.94
C : T	+0.88	+0.84	+0.87	+0.90
A : T	+0.89	+0.83	+0.76	+0.70
n	11	13	11	10
V (=n-2)	9	11	9	8

(b) Trial 2

Pair	Day 2	Day 3	Day 4	Day 5	Day 6
C : A	+0.89	+0.85	+0.86	+0.85	+0.86
C : T	+0.87	+0.77	+0.88	+0.81	+0.86
A : T	+0.88	+0.84	+0.86	+0.76	+0.84
n	10	9	9	6	7
V (=n-2)	8	7	7	4	5

(c) Trial 3

Pair	Day 2	Day 3	Day 4	Day 5
C : A	+0.88	+0.85	+0.91	+0.92
C : T	+0.88	+0.84	+0.88	+0.90
A : T	+0.87	+0.85	+0.74	+0.62
n	24	20	21	17
V (=n-2)	22	18	19	15

(Hayslett & Murray, 1974.)

TABLE 19

Estimates of the standard errors of correlation for the three category rating experiments (see Table 18)

$$\text{Standard error} = \sigma\sqrt{1 - (r^2)}$$

(a) Trial 1

Pair	Day 2	Day 3	Day 4	Day 5
C : A	±1.36	±1.45	±0.63	±0.55
C : T	±1.36	±1.49	±0.74	±0.74
A : T	±0.59	±0.57	±0.88	±0.67

(b) Trial 2

Pair	Day 1	Day 3	Day 4	Day 5	Day 6
C : A	±0.48	±0.73	±0.88	±0.43	±0.64
C : T	±0.52	±0.89	±0.82	±0.48	±0.65
A : T	±0.71	±0.75	±0.83	±0.90	±1.08

(c) Trial 3

Pair	Day 2	Day 3	Day 4	Day 5
C : A	±0.42	±0.53	±0.52	±0.69
C : T	±0.42	±0.59	±0.60	±0.76
A : T	±0.61	±1.02	±0.95	±1.07

(Hayslett & Murray, 1974.)

TABLE 20

Pooled estimates of correlation coefficient (r) by Fisher's Z-transformation

Trial 1

	r	S.E.
Colour v. Aroma	+0.89	±0.15
Colour v. Taste	+0.87	±0.45
Aroma v. Taste	+0.80	±0.15

Trial 2

	r	S.E.
Colour v. Aroma	+0.85	±0.16
Colour v. Taste	+0.84	±0.16
Aroma v. Taste	+0.87	±0.16

Trial 3

	r	S.E.
Colour v. Aroma	+0.89	±0.12
Colour v. Taste	+0.88	±0.12
Aroma v. Taste	+0.80	±0.12

All attributes

	r	S.E.
Trial 1	+0.86	±0.09
Trial 2	+0.85	±0.09
Trial 3	+0.86	±0.07

Table 20 (Cont/.)

TABLE 20 (Continuation)

All Trials

	r	S.E.
Colour v. Aroma	+0.87	±0.08
Colour v. Taste	+0.87	±0.08
Aroma v. Taste	+0.82	±0.08

(Moroney, I973.)

TABLE 21

Analysis of variances for Trial 1 ; values for F

(a) Appearance of raw sausages (all values)

Source of variance	Sum of squares	V	o^2	F
Between samples	13.80	3	4.36	2.61
Within samples	68.46	41	1.67	
Total				

(b) Aroma of raw sausages (all values)

Source of variance	Sum of squares	V	o^2	F
Between samples	129.35	3	43.28	17.46
Within samples	101.61	41	2.48	
Total				

(c) Flavour of cooked sausages (all values)

Source of variance	Sum of squares	V	o^2	F
Between samples	100.2	3	33.4	13.2
Within samples	103.13	41	2.51	
Total				

(Moroney, I973.)

TABLE 22

Analysis of variance for Trial 1 : values for 'F'

(a) Appearance of raw sausages (days 2, 3 only)

Source of variance	Sum of squares	V	σ^2	F
Between samples	7.145	1	7.145	8.13
Within samples	19.333	22	0.88	
Total				

(b) Aroma of raw sausages (days 2, 3 only)

Source of variance	Sum of squares	V	σ^2	F
Between samples	60.85	1	60.85	49.25
Within samples	27.18	22	1.24	
Total				

(c) Taste of cooked sausages (days 2, 3 only)

Source of variance	Sum of Squares	V	σ^2	F
Between samples	34.43	1	34.43	17.5
Within samples	43.26	22	1.96	
Total				

TABLE 23

Analysis of variance for Trial 2 : values for 'F'

(a) Appearance of raw sausages containing sulphur dioxide (all values)

Source of variance	Sum of squares	V	o^2	F
Between samples	19.162	4	4.79	
Within samples	62.26	36	1.73	+2.77
Total	81.42	40		

(b) Aroma of raw sausages containing sulphur dioxide (all values)

Source of variance	Sum of squares	V	o^2	F
Between samples	57.19	4	14.30	
Within samples	83.73	36	2.33	+6.14
Total	140.93			

(c) Taste of cooked sausages containing sulphur dioxide (all values)

Source of variance	Sum of squares	V	o^2	F
Between samples	27.17	4	6.79	
Within samples	54.32	36	1.51	+4.50
Total	81.49			

TABLE 24

Analyses of variance for Trial 2 : values for 'F'
(days 1 and 4 only)

(a) Appearance of raw sausages containing sulphur dioxide

The results for variance analysis on days 1 to 4 inclusive do not demonstrate a significant difference to the results for all the values taken. No table is therefore given. At best, F < +1.97.

(b) Aroma of raw sausages containing sulphur dioxide

Source of variance	Sum of squares	V	o^2	F
Between samples	8.69	2	4.345	
Within samples	50.54	25	2.022	+2.149
Total	59.23			

(c) Taste of cooked sausages containing sulphur dioxide

Source of variance	Sum of squares	V	o^2	F
Between samples	15.98	2	7.99	
Within samples	38.08	25	1.52	+5.26
Total	54.06			

TABLE 25

Analysis of variance for Trial 3 : values for 'F'

(a) Appearance of raw sausages (all trials)

Source of variance	Sum of squares	V	o^2	F
Between samples	167.44	3	55.81	9.81
Within samples	444.42	78	5.69	
Total	611.86			

(b) Aroma of raw sausages (all trials)

Source of variance	Sum of squares	V	o^2	F
Between samples	241.67	3	80.56	43.55
Within samples	142.51	77	1.85	
Total	384.18			

(c) Flavour of cooked sausages (all values)

Source of variance	Sum of squares	V	o^2	F
Between samples	31.20	3	10.40	6.75
Within samples	120.37	78	1.54	
Total	151.57			

TABLE 26

Analysis of variance for Trial 3 : values for 'F'

(a) Appearance of raw sausages (day 2, 3 only)

Source of variance	Sum of squares	V	o^2	F
Between samples	57.76	1	57.76	24.27
Within samples	100.07	42	2.38	
Total	157.83			

(b) Aroma of raw sausages (day 2, 3 only)

Source of variance	Sum of squares	V	o^2	F
Between samples	10.86	1	10.86	5.87
Within samples	66.56	36	1.85	
Total	77.42			

(c) Flavour of cooked sausages (day 2, 3 only)

Source of variance	Sum of squares	V	o^2	F
Between samples	21.64	1	21.64	22.48
Within samples	40.42	42	0.93	
Total	62.06			

TABLE 27

Comparative analyses of variances of the three attributes (all values)

Colour

	Trial 1	Trial 2	Trial 3
F	2.61	2.77	9.81
ν_1	3	4	3
ν_2	4	36	78

Aroma

	Trial 1	Trial 2	Trial 3
F	17.5	6.14	43.6
ν_1	3	4	3
ν_2	41	36	78

Taste

	Trial 1	Trial 2	Trial 3
F	13.2	4.50	6.75
ν_1	3	4	3
ν_2	41	36	78

F : Snedecor's F Statistic

ν_1 : Degree of freedom between samples ($n_1 - 1$)

ν_2 : Degree of freedom within samples ($n_2 - 1$)

TABLE 28

Trial 1 : estimates of significance of F values at four probability levels, for three stimuli and two comparisons

α	Analysis	Stimulus	ν_1	ν_2	F Pred	F Calc	$F_C - F_P$ +
0.05	All values	Appearance	3	41	2.84	2.61	-0.23
		Aroma	3	41	2.84	17.46	+14.62
		Taste	3	41	2.84	13.20	+10.36
	Days 2 & 3	Appearance	1	22	4.30	8.13	+3.83
		Aroma	1	22	4.30	49.25	+44.95
		Taste	1	22	4.30	17.50	+13.20
0.025	All values	Appearance	3	41	3.46	2.61	-0.85
		Aroma	3	41	3.46	17.46	+14.0
		Taste	3	41	3.46	13.20	+9.74
	Days 2 & 3	Appearance	1	22	5.79	8.13	+2.34
		Aroma	1	22	5.79	49.25	+43.46
		Taste	1	22	5.79	17.50	+11.71
0.01	All values	Appearance	3	41	4.31	2.61	-1.7
		Aroma	3	41	4.31	17.46	+13.15
		Taste	3	41	4.31	13.20	+8.89
	Days 2 & 3	Appearance	1	22	7.95	8.13	+0.18
		Aroma	1	22	7.95	49.25	+41.30
		Taste	1	22	7.95	17.50	+9.55
0.005	All values	Appearance	3	41	6.59	2.61	-3.98
		Aroma	3	41	6.59	17.46	+10.89
		Taste	3	41	6.59	13.20	+6.61
	Days 2 & 3	Appearance	1	22	14.38	8.13	-6.25
		Aroma	1	22	14.38	49.25	+34.87
		Taste	1	22	14.38	17.50	+3.12

+ Positive values for ($F_{Cal} - F_{Pred}$) are significant

TABLE 29

Trial 2 : estimates of significance of F values at four probability levels, for three stimuli and two comparisons

α	Analysis	Stimulus	ν_1	ν_2	F_{Pred}	F_{Calc}	+Difference
0.05	All values	Appearance	4	36	2.65	2.77	+0.12
		Aroma	4	36	2.65	6.14	+3.49
		Taste	4	36	2.65	4.50	+1.85
	Days 1-4 only	Appearance	2	25	3.39	1.90	-1.49
		Aroma	2	25	3.39	2.15	-1.24
		Taste	2	25	3.39	5.26	+1.87
0.025	All values	Appearance	4	36	3.19	2.77	-0.42
		Aroma	4	36	3.19	6.;4	+2.95
		Taste	4	36	3.19	4.50	+1.31
	Days 1-4 only	Appearance	2	25	4.30	1.90	-2.40
		Aroma	2	25	4.30	2.15	-2.15
		Taste	2	25	4.30	5.26	+0.96
0.010	All values	Appearance	4	36	3.93	2.77	-1.16
		Aroma	4	36	3.93	6.14	+2.21
		Taste	4	36	3.93	4.50	+0.57
	Days 1-4 only	Appearance	2	25	5.57	1.90	-3.67
		Aroma	2	25	5.57	2.15	-3.42
		Taste	2	25	5.57	5.26	-0.31
0.005	All values	Appearance	4	36	5.91	2.77	-3.14
		Aroma	4	36	5.91	6.14	+0.23
		Taste	4	36	5.91	4.50	-1.41
	Days 1-4 only	Appearance	2	25	9.23	1.90	-7.33
		Aroma	2	25	9.23	2.15	-7.08
		Taste	2	25	9.23	5.26	-3.97

+Positive values for ($F_{Calculated} - F_{Predicted}$) are significant

TABLE 30

Trial 3 : estimates of significance of F values at four probability levels, for three stimuli and two comparisons

α	Analysis	Stimulus	ν_1	ν_2	F_{Pred}	F_{Calc}	$F_C - F_P$ +
0.05	All values	Appearance	3	78	2.76	9.81	+7.05
		Aroma	3	77	2.76	43.55	+40.79
		Taste	3	78	2.76	6.75	+3.99
	Days 2,3	Appearance	1	42	4.08	24.27	+20.19
		Aroma	1	36	4.12	5.87	+1.75
		Taste	1	42	4.08	22.48	+18.4
0.025	All values	Appearance	3	78	3.34	9.81	+6.47
		Aroma	3	77	3.34	43.55	+40.21
		Taste	3	78	3.34	6.75	+3.41
	Days 2,3	Appearance	1	42	5.42	24.27	+18.85
		Aroma	1	36	5.50	5.87	+0.37
		Taste	1	42	5.42	22.48	+17.05
0.010	All values	Appearance	3	78	4.13	9.81	+5.68
		Aroma	3	77	4.13	43.55	+39.42
		Taste	3	78	4.13	6.75	+2.62
	Days 2,3	Appearance	1	42	7.31	24.27	+16.96
		Aroma	1	36	7.43	5.87	-1.56
		Taste	1	42	7.31	22.48	+15.17
0.005	All values	Appearance	3	78	6.17	9.81	+3.64
		Aroma	3	77	6.17	43.55	+37.38
		Taste	3	78	6.17	6.75	+0.58
	Days 2,3	Appearance	1	42	12.61	24.27	+11.66
		Aroma	1	36	12.95	5.87	-7.08
		Taste	1	42	12.61	22.48	+9.87

+Positive values for (F_{Calc} - F_{Pred}) are significant)

TABLE 31

Partial regression equations (obtained by multivariate regression analysis) expressing each attribute in terms of the other two, with correlation coefficients (r) and standard deviations (S)

COLOUR (X)

Trial 1	X=0.302Y+0.192Z+3.24	r=+0.85	S=±0.79	n=45
Trial 2	X=0.426X+0.258Z+1.25	r++0.86	S=±0.93	n=41
Trial 3	X=0.048Y+0.507Z+2.88	r=+0.89	S=±1.87	n=82

AROMA (Y)

Trial 1	Y=0.233X+0.358Z+1.15	r=+0.85	S=±2.76	n=45
Trial 2	Y=0.514X+0.270Z+0.722	r=0.85	S=±2.20	n=41
Trial 3	Y=0.070X+0.480Z+1.64	r=+0.84	S=±3.59	n=82

TASTE (Z)

Trial 1	Z=0.527X+0.348Y+0.50	r=+0.86	S=±1.02	n=45
Trial 2	Z=0.254X+0.327Y+2.45	r=+0.87	S=±1.45	n=41
Trial 3	Z=0.537X+0.451Y-0.007	r=+0.80	S=±5.12	n=82

(Moroney, I973.)

observation that the aroma of the raw sausage is probably the most sensitive sensory indicator of the three studied, this was expected.

Estimates of each unknown, calculated using these equations, agreed quite closely with the observed values.

2. Graphical rating experiment

This experiment was intended to confirm the results obtained using the hedonic scaling techniques previously described; by changing the format of the scoring system and using a non-category scale to reduce bias in the results, a better and more sensitive indication of the changes in the aroma qualities of raw sausages was sought (Tables 32-34).

Variance analysis was again used to confirm an observation that there was a sudden change in the mean score for the aroma after the fourth day. For the full range sample (production to termination of the experiment), $F = +9.77$ which, for $\nu_1 = 5$, $\nu_2 = 94$ degrees of freedom, exceeds the predicted value of ± 4.67 (for a type-1 probability error of $\alpha = 0.01$), indicating significant between-sample variation. For the sub-sample (production to day 4) $F = +0.8$. At $\nu_1 = 3$, $\nu_2 = 57$, and the same error probability, the predicted value of F was ± 4.31. The calculated value does not fall outside the limiting

range, and therefore it is assumed that no significant 'between-sample' variation occurred. The change in the value of F was concluded to be due to a significant change in the mean scores for the aroma after the fourth day of incubation.

The confidence in this hypothesis was estimated by calculating Student's t. The grand average score for the first four days was 83.78. A hypothesis was formulated that this was the true average response for the aroma character, and the null hypothesis $H_0 : \mu_0 = 83.78$ was tested against the alternative $H_1 : \mu_0 \neq 83.78$ for the individual average responses recorded on each of the six days of the trial, using the expression:

$$t = \frac{\bar{x} - \mu_0}{s_w \sqrt{n}}$$

Equation IV

(Hayslett & Murray, 1974.)

The results of the calculation showed that the hypothesis $H_0 : \mu_1 = +83.78$ is acceptable for days 1 to 4 (as must be expected) but is unacceptable for days 5 and 6. The probability of committing an error in accepting this observation as correct when it is in fact false, is not greater than 0.0005.

The results indicated that there was fairly strong evidence to suggest that a sudden change in the acceptability of the aroma of the sausage occurred between the fourth and fifth day of storage at ca. 20°C.

Observations recorded during the qualitative experiments suggested that some components of the aroma (e.g. herbs, and sulphur dioxide) disappeared as the shelf life progressed. The results of the graph rating experiment were analysed to determine if this affected the acceptability of the sausage aroma during the four days of storage at 20°C between manufacture, and the sudden onset of unacceptable deterioration. Two estimates of lines of best fit (derived by the least-mean squares technique) were calculated:

(a) $y = 2.672x + 77.61$

(b) $\log_{10} y = 0.0142x + 1.8895$

where x : age of the sausage in days.

y : mean odour response.

Expression (a) is a linear function derived from the existing information. In expression (b) the aroma scores were transformed to $\log_{10}$; from the literature survey of the psychophysics of sensory testing, an exponential relationship was considered a possibility (p.31).

The suitability of (a) and (b) as true expressions of the relationship between aroma score and time was tested, in terms of their respective linearities. Expressing the slope of the regression line as β, the null hypothesis

was proposed that $\beta = 0$ (against $H_1 : \beta \neq 0$) in the equation:

$$t = \frac{b - \beta_0}{\sqrt{\frac{\sum(y_i - \hat{y}_i)^2}{n - 2}}}$$

Equation V

(Hayslett & Murray, I974.)

The predicted value for t at $\beta = 0.05$ and $\nu = 2$ degrees of freedom is $\pm$ 2.92. Equation (a) yielded $\pm$ 1.51 and (b) $\pm$ 1.09. Both fell within the critical range, and therefore the null hypothesis was assumed correct - neither of the two linear expressions could be confidently regarded as good estimates of any underlying trend. The conclusion was drawn that, for the number of results observed, the change in magnitude of the aroma score was not sufficiently large to indicate a distinct decline in quality, nor was the difference in mean scores sufficiently great and consistent to indicate a specific change. The distribution of the results for the first four days was thought to be caused by residual 'time-order' and 'hysteresis' errors (p. 29). The aroma quality of raw sausages stored at ca. 20-22°C therefore does not significantly change, and does not become unacceptable, until ca. 4 to 5 days after manufacture, when it deteriorates suddenly. This event coincides with other climax phenomena, to be described. It is noteworthy that the standard deviations of the means of the observed values (Table 32) increase up to the fourth day, then decrease again. This is consistent with the prediction from organoleptic

theory (p.29) that at low concentrations and at positions on a scale near to the neutral point, time-order effects are a source of error. This is caused by difficulties in giving a preference rating to a bland product (blandness being a feature of the modern sausage - p.6), compared to the distinctive and recognisable characteristics of the spoiled product.

TABLES 32-34

Table 32 : Results of graph rating experiment for aroma

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Mean	77.06	90.1	81.0	89.0	122.5	119
S.D.	$\pm$22.79	$\pm$22.77	$\pm$29.16	$\pm$35.58	$\pm$27.80	$\pm$20.02
n	16	15	18	12	19	15
D.F(n-1)	15	14	17	11	18	14
Σx	1233	1352	1458	1068	2328	1789
Σx^2	102806	129027	157877	108978	299156	218858

Table 33 : Analysis of data for covariance (all values)

Source of variation	Sum of squares	D.F. (n-1)	Variance estimate
Between samples	32228	5	6446
Within sample	61985	94	659

F = 9.77

Table 34 : Analysis of covariance (Days 1-4 only)

Source of variation	Sum of squares	D.F. (n-1)	Variance estimate
Between samples	1788	3	596
Within sample	42465	57	745

F = 0.80

SAUSAGE MICROBIOLOGY

The two previous sections were concerned with time dependent quantitative changes in the organoleptic quality of sausages, and the effect of the availability of glucose (as determined by carbohydrate source, or the presence of antimicrobial additives) on qualitative changes. The results implicated the availability of glucose as a determinant of the magnitude of organoleptic change in sulphited sausages, and sulphur dioxide as the cause of organoleptic stability.

Substrate levels of glucose have been demonstrated in sausages (Abbiss 1978) and are thought to be a factor contributing to the growth of bacterial and yeast contaminants. The composition and development of this microbial association has been investigated (Dowdell and Board 1968, Hurst 1972, Brown 1977, Abbiss 1978) and described in the introduction to this study (p. 33). Domination by the bacterium Microbacterium thermosphactum is most common, although domination by yeasts is occasionally observed (Brown 1977, Abbiss 1978). In the former instance, climax populations of 10^8 - 10^9 c.f.u./g. have been reported (Brown 1977, Abbiss 1978). These authors have also noted that sulphiting has little effect on growth and the climax population.

The spoilage of meat and meat products has generally been associated with the growth of bacteria (Ingram and Dainty 1971), and noticeable organoleptic changes are reported to

occur at levels of 10^8/g. The climax population density in the sausage might therefore be large enough to cause spoilage per se. If this is so, then the rapidity with which the association develops to its climax might determine shelf-life. Similarly, if glucose constitutes a fermentable energy source for the flora, then its availability could constitute a limitation to growth. The aim of this section is to relate the organoleptic changes previously described to observations on the composition of the microbial association under varying conditions of glucose availability, and in the presence of different antimicrobial additives added as alternatives to SO_2.

1. General observations on the composition of the microbial association

   The distribution of the components of the microbial association, as elicited by enumeration on elective and selective media (p. 66) and by microscopic examination of slides prepared from the total flora samples (SPC agar + 0.2% NaCl; p.66), was as expected for a bacterially dominated sausage (Brown 1977, Abbiss 1978); Microbacterium thermosphactum dominated, with Pseudomonas spp., yeasts and Lactobacillus spp. present. Micrococcus spp. could also be recovered from SPC agar plates, and with yeasts and Microbacterium thermosphactum constituted the three most common types of organism occurring on this general plating medium.

   Footnote : SPC - Standard Plate Count Agar

The substitution of alternative carbohydrates for sausage rusk in sulphited sausages affected the size and rate of development of the association, but did not significantly affect the proportions of the major components. In the sulphited cellulose, and the sulphited flour, sausages, Micrococcus spp. were noticeably more common on SPC agar plates than the other two groups described above. (Micro-colonies of this organism were also plentiful on the exterior of sausages containing flour, p.123 . The dominance of Micrococcus spp. in the flora of raw flour might explain this latter observation.

The principal effect of the omission of sulphite was the growth of coliform organisms; these were Gram-negative cocco-bacilli tolerant of Violet-Red Bile Agar. In the presence of the preservative they could not be detected without prior enrichment.

The inclusion of alternative antimicrobials to SO_2 produced more specific effects during the first four days of storage. Sodium arsenite did not produce a change in the general composition of the association, although it did have an effect in retarding the growth of the Pseudomonas population (to be described). Overall, sulphite and arsenite appeared interchangeable. Sodium fluoride, alone or in the presence of either sulphite or arsenite, caused repression of the growth of the association, in whole or in part (to be described).

Colonies grown from sausages containing this antimicrobial were of pinpoint size on SPC agar and Gardner's medium (p. 66). Microscopic examination of slides prepared from the latter showed that Microbacterium thermosphactum seemed to be in its coccoid form.

Colonies grown from sausages containing 2 : 4 : dinitrophenol were large and mucoid on SPC agar, had a Gram-positive coccoid morphology, and gave off a sweet, sickly aroma.

2. The development of the microbial association in the sulphited sausage at room temperature

All experiments conducted in this study included a control, comprising a standard pork sausage containing rusk and sulphur dioxide. The microbiological data from the controls were pooled, and for each data point the mean value and standard deviation was calculated (Table 35). The averaged growth curves of the components of this control flora at the outer surface and the core locations are illustrated in Fig. 16.

The initial rates of growth (from day 1 to day 4) of the flora components were calculated using the method of least-mean-squares, and the confidence in the lines estimated from their coefficients of correlation (Table 36). Comparisons were subsequently made of the regression line slopes by calculating Student's 't' from the equation:-

$$t = \frac{(m - m_0)\sqrt{N - 2}}{\sqrt{1 - r^2}}$$

where m = slope of regression line for group studied

m_0 = slope of regression line for TVC

r = correlation coefficient, $m : m_0$

N = number of data pairs in sample

(ref. Moroney 1973)

The populations of the total mixed flora (TVC), Microbacterium thermosphactum, and Pseudomonas spp. were observed to climax at the surface sampling location between the fourth and fifth day after manufacture, if the product was first refrigerated for 24 hours and subsequently stored at 20-22°C. At the core location, the same phenomenon was observed, although the climax populations were smaller by about one decade for the total mixed population, and about one and a half decades for the other populations.

Populations of yeasts and Lactobacillus spp. did not climax at the same time as the others, but continued growing. There is a suggestion that the yeast growth curve rate decreased at the time of the association's climax, but it has not been observed to become negative. Lactobacillus spp. continued to increase in number without apparent check, and at a constant rate.

The pooled, averaged values for the populations at the surface and core locations of the sausage differed by 1.0 - 1.5 decades. The difference was found to be significant at $P_0 = 0.1$ for all the values studied (Table 37) using Student's 't', as calculated from the equation:

$$t = \frac{|\bar{X} - \bar{x}|}{s} \times \sqrt{n - 1} \qquad \text{(Moroney 1973)}$$

where $|\bar{X} - \bar{x}|$ = difference between the means of the sample and the population

n = number of paired comparisons

s = standard deviation of the differences between the paired comparisons

Using 't' as an in indicator of the effect of sample location, it was noted that the Pseudomonas spp. were affected the most, and yeasts the least. Lactobacillus spp. also did not seem to be affected by the origin of the sample (construed to be an effect of oxygen availability) but their mean populations values were small in relation to the total population, and their standard deviations very large. It was concluded that the counting efficiency of this medium was poor.

Comparison of the values of m (for each population component) with m_0 (TVC) showed that (Table 38) the initial rate of growth of the yeast population was significantly different, and faster, than the other

population components.

Conversely, the Lactobacillus population had a value of 't' significant but negative; this group of organisms might not therefore be able to compete with the other components of the population during the growth phase of the association.

A comparison was also made between growth of the two largest bacterial populations (Microbacterium thermosphactum and Pseudomonas spp.) and yeasts. At the outer surface, significant differences were noted, with the yeast : M. thermosphactum growth rate comparison being the most significant, and greater than the yeast : Pseudomonas spp. comparison, which in turn was more significant than yeast : TVC. At the core location, a significant comparison was obtained for yeast : Pseudomonas spp.

The dominance of yeasts as a group late in the shelf-life of the sausage may therefore be explained on the basis of superior growth rate, when compared to both the TVC, and specific competitors (Microbacterium thermosphactum and Pseudomonas spp.). In a normal bacterially dominated sausage, with an initial population of ca. 10^5 c.f.u./g., yeasts will assume dominance only when the bacterial populations have climaxed (Brown 1977, Abbiss 1978). In sausages with a lower initial TVC (e.g. 10^4 c.f.u./g.) the superior growth rate of

yeasts might give them a competitive advantage, and permit their early population dominance.

3. The effect of alternatives to sausage rusk in the presence of sulphur dioxide on the established population profile

Data from experiments in which raw flour, heat-treated raw flour, yeast-leavened breadcrumb, rusk supplemented with thiamine HCl (450mgs/kg of sausage), cellulose, cellulose and glucose at 0.1%, and cellulose and glucose at 1%, were also analysed for initial rate of growth, using the least-mean-squares technique (Tables 39 and 40).

Raw flour gave the most significant differences observed, at both sample locations. It particularly stimulated Microbacterium thermosphactum. Cellulose also significantly stimulated the Pseudomonas population at the surface location for co-dominance of the flora. Adding glucose back to this latter formulation had an interesting effect; at 0.1%, the inhibitory effect of cellulose on Microbacterium thermosphactum was reversed, and a stimulation of rate was observed in excess of, for example, breadcrumb. Increasing the inclusion to 1%, however, produced inhibition of this group, and the rest of the flora. Results reported in the next section will show that this might be caused by an increased H^+ ion accumulation, perhaps caused by the action of meat enzymes.

In general, good correlation with the rusk control was observed for populations of micro-organisms growing at the surface location in the various sausage formulations. The correlations for the core locations, when compared with the control, were very poor, except for rusk supplemented with thiamine at 450mgs/kg. This is because none of the values for r for comparisons made between samples from this location exceeded $\pm$ 0.9, the statistical limit for the number of samples taken. The corresponding comparisons made at the surface location all correlate with a significance of at least P = 0.1 for their regression-line slopes, under the same conditions.

4. The effect of sulphur dioxide

Experimental evidence was obtained which supported the results of Brown (1977) and Abbiss (1978) that the flora of unsulphited sausages, with the exception of the presence of coliforms, is very similar to that of the sulphited product (Fig. 16 - overlay). No statistically significant differences in the two floras could be found in the results obtained in this study. For clarity, the results from these experiments are tabulated in Tables 40 and 41, and included in the section on alternative antimicrobials.

5. The effects of alternative antimicrobials to SO_2

Evaluation of the initial rates of growth of the main components of the association (TVC, Microbacterium

thermosphactum and Pseudomonas spp.) at the outer surface location showed that significant decreases in rates were caused by some of the antimicrobials when compared to SO_2 (Tables 41, 42 and 43). Most of the antimicrobial effects observed affected the TVC or Pseudomonas components; Microbacterium thermosphactum seemed to be relatively resistant to their effects (Table 43). The only antimicrobials which affected the dominant organism were combinations which included fluoride. Arsenite and sulphite appeared interchangeable in respect of their action on this microorganism, and the addition of fluoride to either produced an increase in antimicrobial effect. Fluoride + DNP gave a similar value for 't' as F + SO_2; however, as previously recorded, there was a difference in colony morphology.

6. Estimates of initial rate and population maxima

In the postulated regression equation $\log_{10}y = mx + c$, m represents the rate of change of the population ($\log_{10}y$) with time (x) - i.e. the initial rate of growth, and c the population at 24 hours after manufacture. From all the values recorded for the standard sausage, and sausages containing alternative fillers which gave populations equivalent to, and not statistically different from, the control, graphs were plotted (for the two sample locations) of values of m against values of c (Fig. 17). Lines of best fit were estimated by the method of least-mean-squares, and from their

equations, values for the maximum growth rate for an unspecified component of a mixed population, and the maximum density for a mixed population, were calculated. The latter, for the surface location, was $10^{8.71}$ c.f.u./g. (i.e. 5×10^8) and for the core location 10^6 c.f.u./g. The maximum estimate of m was 0.8 log cycles/day for a core population, and ca. 1.5 log cycles/day for a surface population. The rate recorded for the surface is of the order of magnitude observed for yeasts (p.139). The theoretical maximum proposed for this location also falls within the observed range (8.23 ± 0.67), from the averaged data values.

Differences were observed in the slope of the graphs obtained for the two locations; at the surface the rate decreased by 0.171 log cycles/day per decade increase in population, at the core 0.134.

The results in this section show that there is a connection between the growth of the microbial association, and the availability of glucose, in the sulphited sausage. Raw flour is observed to stimulate the growth of the flora, and in particular Microbacterium thermosphactum; cellulose has the reverse effect. Raw flour contains amylases (Kent-Jones and Amos 1957) and the action of these enzymes on the carbohydrate polymers in rusk will cause the release of glucose. Conversely, the use of cellulose - a non-fermentable polymer - will give rise to early glucose limitation.

The addition of fluoride, chosen for its inhibition of glucose metabolism via the formation of fluorophosphates (Mahler and Cordes 1971), is observed to cause a decrease in the growth of Microbacterium thermosphactum.

In the presence of substrate levels of glucose, (Abbiss 1978) estimates have been made of the theoretical maximum populations achievable (Fig. 17), and these compare well with experimental observations. The effect of increasing population density on rate differs at the two locations, and it is proposed that the relationship at the core location is caused by a double limitation; the first is imposed by oxygen availability, and the second competition with meat enzymes for available substrates. Brown (1977) has observed a similar phenomenon.

Fig16 Growth of the main components of the flora of sulphited sausages [as mean n° c.f.u.'s/g.] at the outer surface [——] & core [——] locations, at room temperature, in comparison with their growth in the unsulphited sausage (transparent overlay)

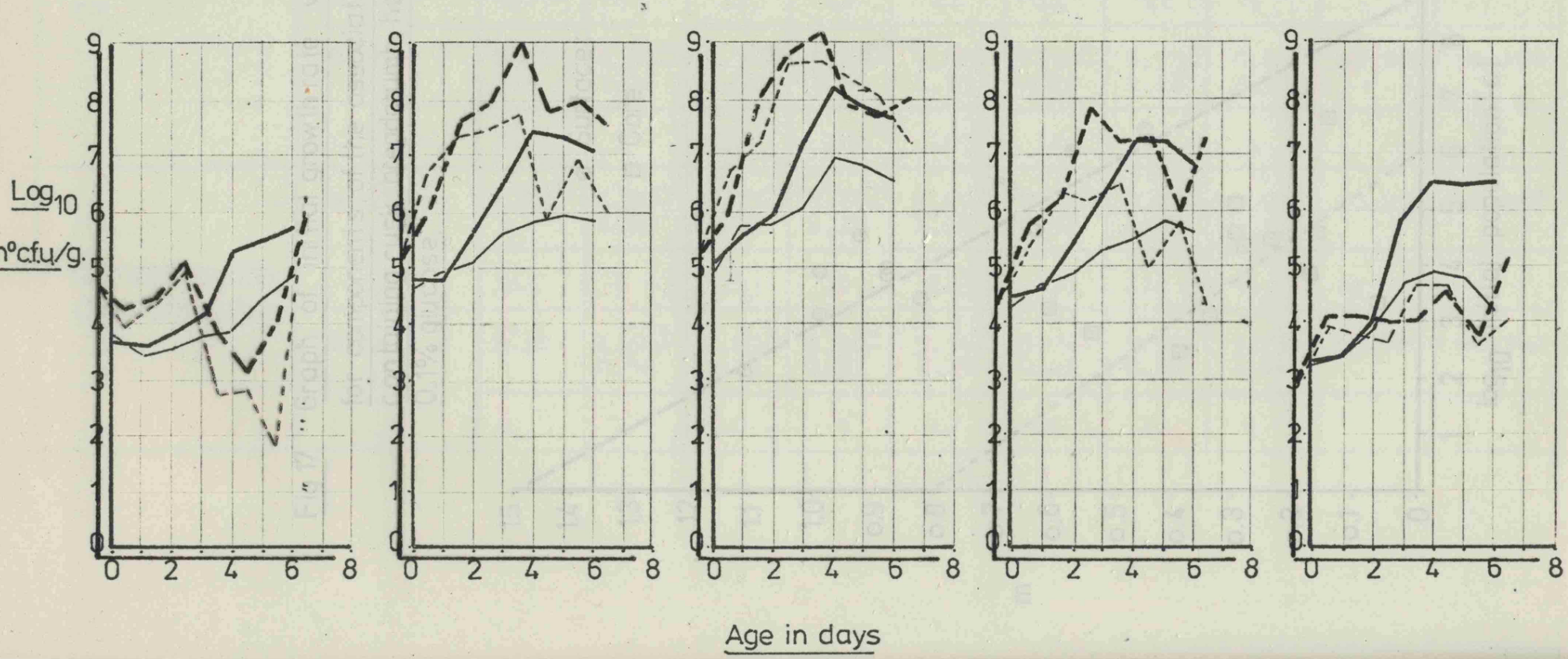


Fig16 Growth of the main components of the flora of sulphited sausages [as mean n° c.f.u.'s/g.] at the outer surface [—] & core [——] locations, at room temperature, in comparison with their growth in the unsulphited sausage (transparent overlay)

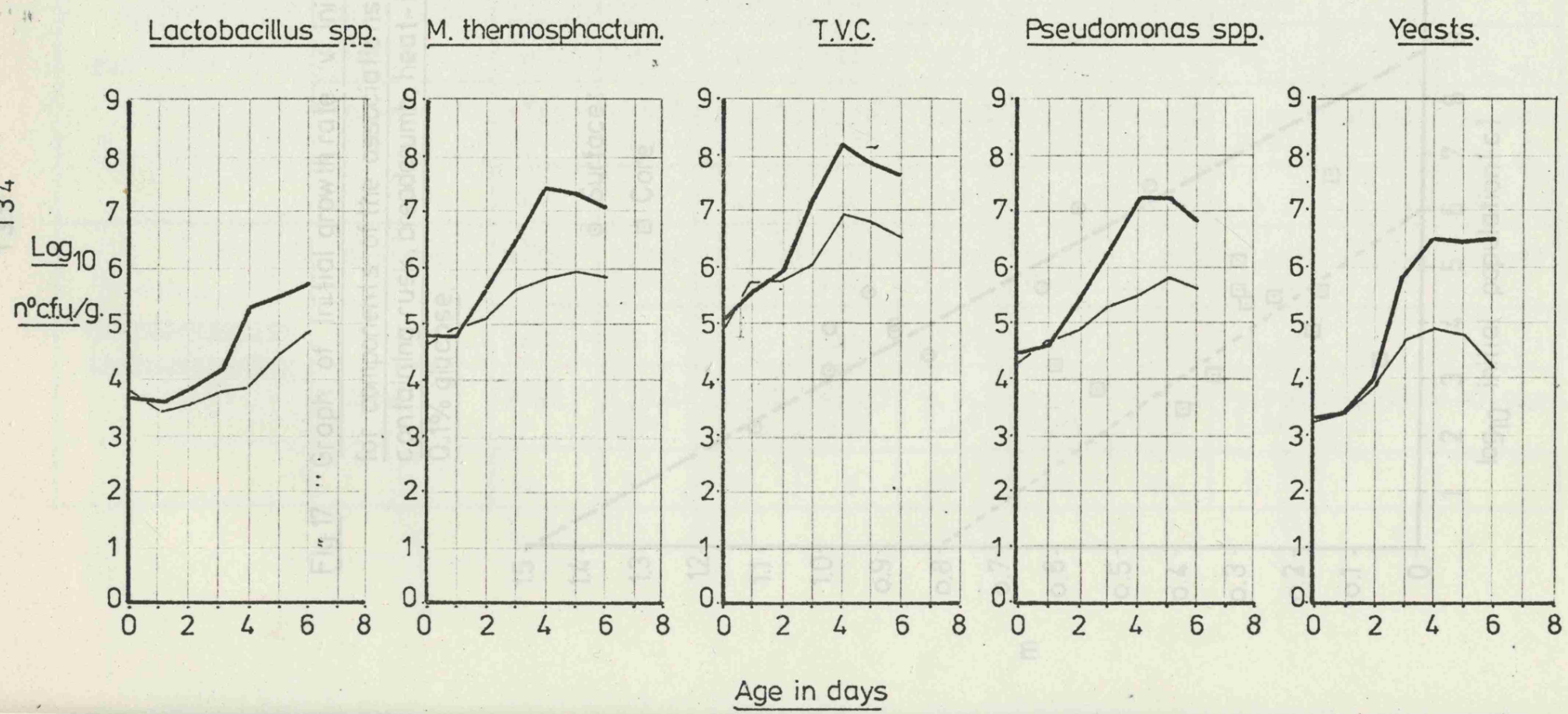


Fig 17 ·· Graph of initial growth rate v. initial population, for components of the associations of sausages containing rusk, breadcrumb, heat-treated flour or 0.1% glucose.

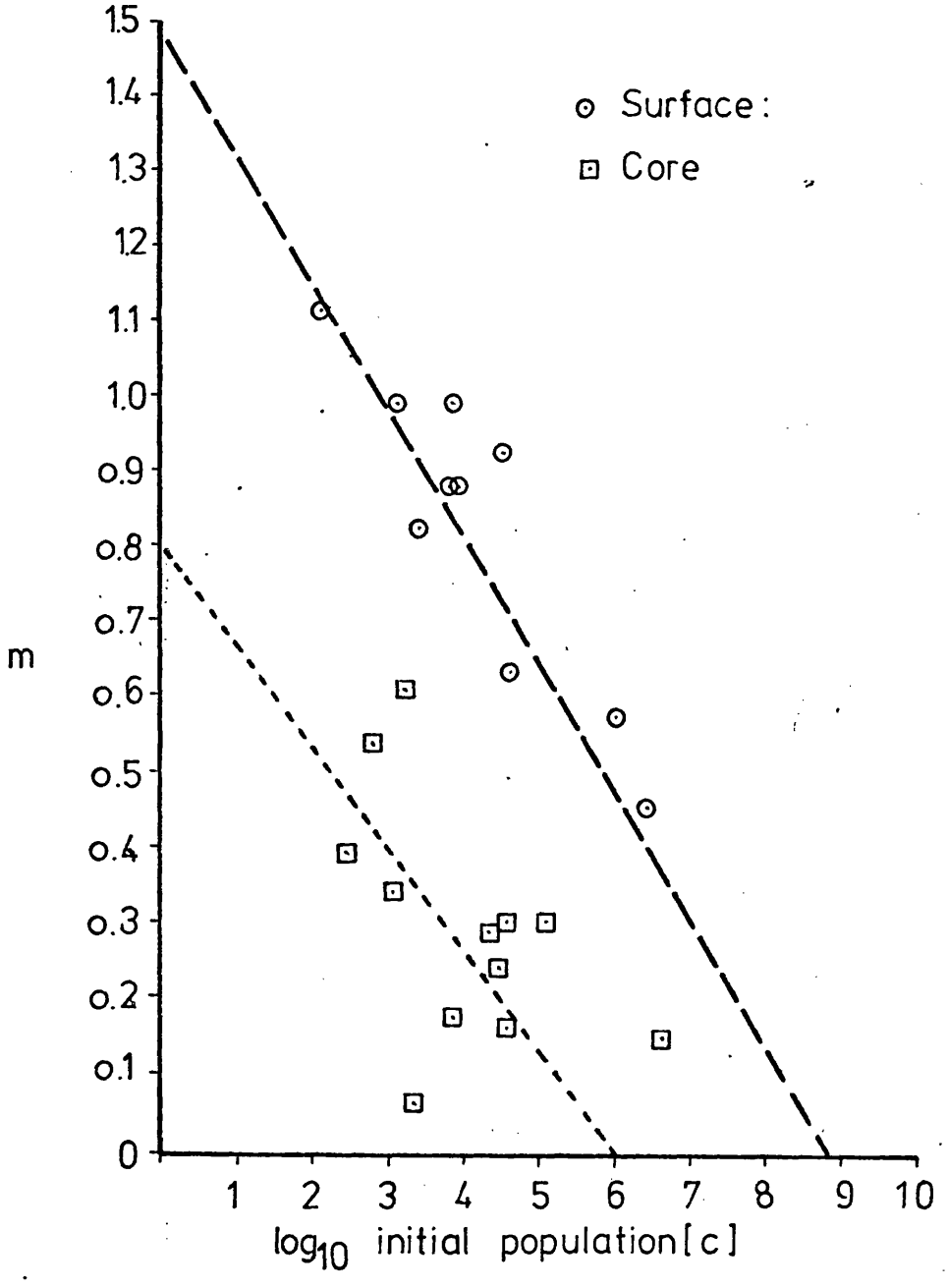


TABLE 35

Averaged results (standard deviations and no. data points) for the major flora components of standard sulphited sausages growing at 20-22°C

Organisms	Location of sample		AGE IN DAYS AT 20-22°C 0	1	2	3	4	5	6	CORRELATIONS V.Time	V.TVC
TVC	Surface	$\bar{x}\pm SD$	5.19 ± 1.48	5.57 ± 1.30	5.91 ± 1.25	7.14 ± 0.93	8.23 ± 0.67	7.88 ± 0.94	7.62 ± 1.26	+0.978	+1.000
		n	8	11	11	11	11	11	4		
	Core	$\bar{x}\pm SD$	4.91 ± 1.36	5.70 ± 0.50	5.72 ± 1.52	6.06 ± 1.15	6.90 ± 1.15	6.75 ± 1.18	6.49 ± 2.30	+0.906	+1.000
		n	7	10	10	10	10	10	4		
Microbacterium thermosphactum	Surface	$\bar{x}\pm SD$	4.77 ± 1.02	4.75 ± 1.19	5.54 ± 1.25	6.47 ± 1.11	7.36 ± 0.93	7.28 ± 1.19	7.02 ± 1.15	+0.999	+0.987
		n	8	11	10	11	11	11	4		
	Core	$\bar{x}\pm SD$	4.60 ± 0.96	4.89 ± 1.19	5.06 ± 1.31	5.54 ± 1.29	5.74 ± 1.45	5.91 ± 0.89	5.79 ± 1.44	+0.981	+0.942
		n	7	10	9	10	10	10	4		

Table 35 (Continuation 1)

Organisms	Location of sample		AGE IN DAYS AT 20-22°C 0	1	2	3	4	5	6	CORRELATIONS V.Time	V.TVC
Pseudomonas spp.	Surface	$\bar{x}\pm SD$	4.45 ± 0.99	4.58 ± 1.15	5.41 ± 1.50	6.28 ± 1.63	7.21 ± 0.80	7.21 ± 0.92	6.80 ± 0.60	+0.999	+0.987
		n	7	10	10	10	10	9	4		
	Core	$\bar{x}\pm SD$	4.25 ± 1.01	4.63 ± 1.21	4.84 ± 1.28	5.25 ± 1.18	5.45 ± 1.16	5.77 ± 0.91	5.57 ± 1.88	+0.990	+0.949
		n	6	9	9	9	9	9	4		
Yeasts	Surface	$\bar{x}\pm SD$	3.28 ± 4.59	3.34 ± 0.44	3.98 ± 0.60	5.79 ± 0.50	6.42 ± 0.61	6.37 ± 1.07	6.43 ± 1.26	+0.978	+0.983
		n	5	8	8	8	8	8	4		
	Core	$\bar{x}\pm SD$	3.19 ± 0.46	3.33 ± 0.70	3.89 ± 0.99	4.63 ± 1.15	4.86 ± 1.15	4.73 ± 0.76	4.14 ± 0.25	+0.982	+0.951
		n	5	8	8	8	8	8	4		

TABLE 35 (Continuation 2)

Organisms	Location of sample		AGE IN DAYS AT 20-22°C 0	1	2	3	4	5	6	CORRELATIONS V.Time	V.TVC
Lactobacillus spp.	Surface	$\bar{x}\pm SD$	3.63 ± 1.18	3.56 ± 1.24	3.78 ± 1.10	4.16 ± 0.95	5.23 ± 1.14	5.45 ± 1.08	5.66 ± 1.47	+0.939	+0.967
		n	6	8	8	8	8	8	3		
	Core	$\bar{x}\pm SD$	3.77 ± 1.27	3.42 ± 0.95	3.56 ± 1.09	3.79 ± 0.02	3.87 ± 0.99	4.43 ± 0.65	4.78 ± 1.14	+0.985	+0.851
		n	5	7	7	6	7	7	4		

TABLE 36

Calculated regression equations and correlations for initial growth rates
(See Table 35)

Group	Location	Regression Equation (Initial Rate, Days 1-4 only)	Correlation Coefficient
TVC	Surface	$\log_{10}Y = 0.921x+4.41$	+0.978
	Core	$\log_{10}Y = 0.394x+5.11$	+0.906
M. Thermosphactum	Surface	$\log_{10}Y = 0.876x+3.84$	+0.999
	Core	$\log_{10}Y = 0.303x+4.55$	+0.981
Pseudomonas spp.	Surface	$\log_{10}Y = 0.876x+3.68$	+0.999
	Core	$\log_{10}Y = 0.287x+4.33$	+0.990
Yeast	Surface	$\log_{10}Y = 1.105x+2.12$	+0.978
	Core	$\log_{10}Y = 0.533x+2.84$	+0.983
Lactobacillus spp.	Surface	$\log_{10}Y = 0.539x+2.84$	+0.939
	Core	$\log_{10}Y = 0.169x+3.25$	+0.976

TABLE 37

Estimate of significance of difference (surface v. core) for organisms from two locations calculated as Student's 't' statistic

		0	1	2	3	4	5	6	t
TVC	Surface	5.19	5.57	5.91	7.14	8.23	7.88	7.62	+3.26
	Core	4.91	5.70	5.72	6.06	6.90	6.75	6.49	
	Difference	+0.28	-0.13	+0.19	+1.08	+1.33	+1.13	+1.13	
M.thermosphactum	Surface	4.77	4.75	5.54	6.47	7.36	7.28	7.02	+3.26
	Core	4.60	4.89	5.06	5.54	5.74	5.91	5.79	
	Difference	+0.17	-0.14	+0.48	+0.93	+1.62	+1.31	+1.23	
Pseudomonas spp.	Surface	4.45	4.58	5.41	6.28	7.21	7.21	6.80	+3.51
	Core	4.25	4.63	4.84	5.05	5.45	5.77	5.57	
	Difference	+0.20	-0.05	+0.57	+1.03	+1.76	+1.44	+1.23	

TABLE 37 (Continuation 1)

		0	1	2	3	4	5	6	t
Yeasts	Surface	3.28	3.34	3.98	5.79	6.42	6.37	6.48	+2.99
	Core	3.19	3.33	3.89	4.63	4.86	4.73	4.14	
	Difference	+0.09	+0.01	+0.10	+1.16	+1.56	+1.64	+1.28	
Lactobacillus spp.	Surface	3.68	3.56	3.78	4.16	5.23	5.43	5.66	+2.69
	Core	3.77	3.42	3.52	3.79	3.81	4.43	4.78	
	Difference	-0.14	+0.14	+0.26	+0.37	+1.42	+1.02	+0.88	

TABLE 38

(1) Comparison of growth rates (from averaged data, for the period from manufacture to Day 4) of the TVC and flora components, at 22°C

		TVC rate mo	Sample rate m	Correlation Coefficient m : mo	Pairs N	Student's t
M. thermosphactum	Surf.	+0.921	+0.876	+0.987	5	-0.486
	Core	+0.394	+0.303	+0.942	5	-0.468
Pseudomonas spp.	Surf.	+0.921	+0.876	+0.987	5	-0.489
	Core	+0.394	+0.287	+0.949	5	-0.586
Yeasts	Surf.	+0.921	+1.105	+0.983	5	+1.731
	Core	+0.394	+0.533	+0.886	5	+0.519
Lactobacillus spp.	Surf.	+0.921	+0.539	+0.953	5	-2.184
	Core	+0.394	+0.162	+0.356	5	-0.430

(2) A similar comparison between the dominant organism (M. thermosphactum) and Yeasts

		M.thermosphactum rate mo	Yeast rate m	Correl. Coeff. n	Pairs N	Student's t
Yeasts	Surf.	+0.876	+1.105	+0.986	5	+2.044
	Core	+0.303	+0.533	+0.886	5	+0.859

TABLE 38 (Continuation 1)

(3) A similar comparison between Pseudomonas spp. and Yeasts

		Pseudomonas rate mo	Yeast rate m	Correlation Coefficient r	Pairs N	Student's t
	Surf.	+0.876	+1.105	+0.982	5	+2.083
	Core	+0.287	+0.533	+0.975	5	+1.933

TABLE 39

A comparison of the effects of alternatives to rusk on the growth rate and initial population of components of the microbial association of the sausage as estimated by regression analysis

(Table shows values for m and c in the equation logY = mx+c, and correlation coefficient of and values, t)

		TVC			M.therm.			Pseud.			Yeast			Lacto.		
		m	c	r	m	c	r	m	c	r	m	c	r	m	c	r
Rusk+SO_2		0.92	4.41	+0.98	0.88	3.84	+0.99	0.88	3.68	+0.99	1.11	2.12	+0.98	0.54	2.84	+0.
		0.39	5.11	+0.91	0.30	4.55	+0.98	0.29	4.33	+0.99	0.53	2.84	+0.98	0.16	3.25	+0.
Breadcrumb + SO_2		0.49	6.34		0.99	3.87		0.85	3.44							
		0.15	6.59		0.49	4.57		0.54	3.33							
Heat-treated flour + SO_2		1.22	2.73		0.36	3.61		1.31	2.65		1.44	2.01				
		0.16	4.51		-0.13	4.60		0.24	4.42		0.62	3.27				
Flour + SO_2		1.42	2.70		1.66	1.75		1.22	2.48							
		0.59	3.94		0.46	3.65		0.25	3.58							
Cellulose + SO_2		0.78	3.43		0.00	4.00		0.00	4.00							
		-0.02	4.35		-0.03	4.20		0.23	4.00							
Cellulose + 0.1% Glucose + SO_2		0.81	3.58	0.68	0.54	3.89	0.82	0.28	2.95	0.42	1.24	1.65	0.99	0.97	1.93	0.
		-0.10	4.84	-0.27	-0.17	4.90	-0.87	0.34	3.04	0.79	0.34	3.03	0.73	0.39	2.53	0.

TABLE 39 (Continuation 1)

	TVC			M.therm.			Pseud.			Yeast			Lacto.		
	m	c	r	m	c	r	m	c	r	m	c	r	m	c	r
Cellulose + 1% Glucose + SO_2	0.71	3.69	0.68	0.36	4.52	0.69	0.63	1.94	0.78	1.34	1.51	0.99	0.97	1.99	0.
	-0.14	4.97	-0.40	-0.25	5.40	-0.74	0.30	2.50	0.78	0.28	3.18	0.57	0.29	2.91	0.
Rusk, 450ppm[6] Thiamine + SO_2	1.48	1.68	0.99	1.64	1.32	0.99				1.32	1.86	0.99			
	0.86	2.18	0.87	0.67	2.57	0.87				0.40	2.78	0.92			

TABLE 40

Significance of difference (Student's 't' estimate) between slopes of regression lines for the major components of the microbial association, for alternatives to sausage rusk

		TVC			M.therm.			Pseud.			Yeast			Lacto.		
		N	r	t	N	r	t	N	r	t	N	r	t	N	r	t
Breadcrumb + SO_2	S	6	0.90	-2.13	4	0.93	0.40	4	0.92	-0.23	-	-	-	-	-	-
	C	6	-0.2	-0.5	4	0.99	5.19	4	0.57	0.42	-	-	-	-	-	-
H.T.Flour + SO_2	S	7	0.98	+3.78	5	0.62	-1.15	5	0.98	+3.52	-	-	-	-	-	-
	C	7	-0.2		5	-0.56	-0.91	5	0.80	-0.13	-	-	-	-	-	-
Flour + SO_2	S	7	0.97	+4.48	5	0.97	5.63	5	0.97	2.40	-	-	-	-	-	-
	C	7	0.97	+0.41	5	0.69	0.37	5	-	-	-	-	-	-	-	-
Cellulose + SO_2	S	7	0.93	-2.09	5	0.99	-3.29	5	0.97	5.11	-	-	-	-	-	-
	C	7	-0.2	-1.74	5	-0.42	-6.82	5	0.82	-0.68	-	-	-	-	-	-
Cell. 0.1% Glucose + SO_2	S	5	0.83	-1.72	5	0.83	2.38	5	-	-	5	0.97	1.44	-	-	-
	C	5	0.24	0.84	5	-0.91	-1.47	5	-	-	5	0.83	-0.42	-	-	-
Cell. 1.0% Glucose + SO_2	S	5	0.83	-0.67	5	0.78	-1.42	5	0.78	-0.67	5	0.99	-18.4	5	0.86	1.48
	C	5	0.81	-1.57	5	0.60	-1.20	5	0.66	0.03	5	0.78	-0.72	5	0.50	0.26

TABLE 40 (Continuation 1)

		TVC			M.therm.			Pseud.			Yeast			Lactobacillus		
		N	r	t	N	r	t	N	r	t	N	r	t	N	r	t
Rusk 480ppm thiamine + SO_2	S	5	0.99	-1.30	5	0.99	-4.03	5	0.78	3.21	5	0.91	0.48	5	-	-
	C	5	0.97	-2.89	5	0.90	0.90	5	-		5	0.88	-0.58	5	-	-

TABLE 41

A comparison of the effects of different metabolic inhibitors in the presence of sausage rusk, on the growth rates and initial populations of components of the microbial association of the sausage, as estimated by regression analysis

(Table shows values for m and c in the equation $\log Y = mx + c$, and the correlation coefficient of count and time values)

		TVC			M.therm.			Pseud.			Yeast.			Lacto.		
		m	c	r	m	c	r	m	c	r	m	c	r	m	c	r
Rusk	S	1.02	5.67	0.94	0.90	5.60	0.96	0.61	5.43	0.83	-0.02	4.38	-0.46	-0.08	4.78	-0.21
	C	0.71	6.33	0.95	0.29	6.84	0.93	0.29	5.58	0.83	0.18	3.76	0.58	-0.32	4.94	-0.45
Rusk + SO_2	S	0.92	4.41	0.98	0.88	3.84	0.99	0.88	3.68	0.99	1.11	2.12	0.98	0.54	2.84	0.94
	C	0.39	5.11	0.91	6.30	4.55	0.98	0.29	4.33	0.99	0.53	2.84	0.98	0.16	3.25	0.98
Rusk + ASO_2	S	0.49	5.55	0.84	0.41	5.40	0.71	0.58	4.10	0.99						
	C															
Rusk + F	S	0.53	5.02	0.92	0.37	4.70	0.80	0.58	4.10	0.89						
	C															
Rusk + DNP	S	0.55	5.72	0.97	0.25	5.79	0.67	0.52	4.21	0.91						
	C															
Rusk + F + SO_2	S	0.29	4.91	0.89	0.001	5.06	0.63	0.70	2.80	0.92						
	C															

TABLE 41 (Continuation 1)

		TVC			M.therm.			Pseud.			Yeast			Lacto.		
		m	c	r	m	c	r	m	c	r	m	c	r	m	c	r
Rusk + F + ASO_2	S C	0.29	4.96	0.58	0.001	5.06	0.23	0.70	2.80	0.99						
Rusk + F + DNP	S C	0.27	4.90	0.51	-0.29	5.26	-0.11	0.47	3.47	0.99						

TABLE 42

Significance of differences (Student's 't' statistic) between the slopes of the regression lines for the major components of the microbial association, for various alternatives to sulphur dioxide

		TVC			M.therm.			Pseud.			Yeasts			Lacto.		
		N	r	t	N	r	t	N	r	t	N	r	t	N	r	t
No SO_2	S	7	0.81	0.36	5	0.93	0.12	5	0.86	-0.90						
	C	7	0.86	1.38	5	0.85	-0.04	5	0.84	0.02						
Rusk + F	S	4	0.95	-1.76	4	0.82	-1.24	4	0.88	-0.89						
	C	-	-	-	-	-	-	-	-	-						
Rusk + ASO_2	S	4	0.85	-1.42	4	0.72	-0.94	4	0.99	-5.27						
	C	-	-	-	-	-	-	-	-	-						
Rusk + DNP	S	4	0.99	-8.6	4	0.68	-1.2	4	0.90	-1.2						
	C	-	-	-	-	-	-	-	-	-						
Rusk + $F+SO_2$	S	4	0.96	-3.25	4	0.65	-1.63	4	0.91	-0.6						
	C	-	-	-	-	-	-	-	-	-						
Rusk + $F+ASO_2$	S	4	0.73	-1.31	4	0.82	-2.18	4	0.99	-1.83						
	C	-	-	-	-	-	-	-	-	-						
Rusk + F+DNP	S	4	0.27	-0.96	4	-0.2	-1.65	4	0.99	-3.3						
	C	-	-	-	-	-	-	-	-	-						

TABLE 43

Comparison of effects of antimicrobials on flora components ranked by value of 't', from information in Table 42

t	Antimicrobial(s) and Organism	Assigned Cause
-8.60	DNP on TVC	Uncoupling of oxidative phosphorylation
-5.27	ASO_2 on Pseudomonas spp.	Respiratory block on respiration of pyruvate/ -oxoglutarate
-3.30	F + DNP on Pseudomonas spp.	Limitation for glucose and uncoupling of oxidative phosphorylation.
-3.25	F + SO_2 on TVC	Limitation for glucose. Effect of SO_2 not known.
-2.18	F + ASO_2 on Microbacterium thermosphactum	Limitation for glucose. Respiratory block for pyruvate/ -oxoglutarate.
-1.83	F + ASO_2 on Pseudomonas spp.	" " " "
-1.76	F on TVC	Limitation for glucose.
-1.68	F + DNP on Microbacterium thermosphactum	Limitation for glucose. Oxidative phosphorylation uncoupled.
-1.63	F + SO_2 on Microbacterium thermosphactum	Limitation for glucose. Effect of SO_2 not known.

BIOCHEMICAL CHANGES

The use of bowl-chopping techniques in sausage manufacture destroys the integrity of the meat tissues, and perhaps also that of cells. It is reasonable to assume that, as a result of this, enzymes of meat origin will be distributed throughout the other ingredients in the sausage. As the mass of meat is much greater than that of the micro-organisms, even at the time of spoilage, the enzymes coming from the meat might be expected to contribute to changes in the sausage, the persistence of this contribution being determined by the rates at which particular enzymes become degraded. Thus, the work of Abbiss (1978) has shown that although two amylases and a maltase of pork decay during the storage of the sausage, their activity is still sufficient to supply the microflora with substrate levels of glucose and maltose.

Conversion of glucose to an acid probably occurs during storage; the development of an acid flavour in spoiled sausages is commonly reported, and the traditional view has always been that sausages 'sour'.

Acids may also be produced from fat, another component of the sausage. Pork depot fats are composed mainly of triglycerides, the hydrolysis of which will yield glycerol and free fatty acids (Mahler and Cordes 1971). The oxidation of free fatty acids by various metabolic processes (Morris 1970) yields low molecular weight organic acids

soluble in the aqueous phase of the sausage. Alternatively, peroxidation of unsaturated acids can occur - either spontaneously or mediated by Pseudomonas spp. (Goldman and Raymond 1952) or Micrococcus spp. (Finnerty et al 1962) - resulting in the formation of carbonyls. The effect of SO_2 on these reactions has been investigated by Brown (1977).

(i) Changes in the hydrogen ion concentration

When studying changes in colour, etc. (p.54-60) many batches of 'control' sausages were analysed for pH. They contained meat, water, rusk, a commercial seasoning and SO_2 at an initial concentration of 450p./10^6. The pH changes in these sausages at the outer surface and core locations are given in Table 44 and illustrated in Fig. 18.

There was a linear relationship between pH and time (at room temperature) for up to four days; thereafter the points become scattered. The pH of the outer surface layer decreased at an average rate of - 0.13 units per day at 22-25°C up to the fourth day. The average core pH changed at a slower rate of - 0.07 units per day. The two sets of data correlate (r = +0.959), but the difference observed in initial rates (0.06 units per day) was not sufficient to be statistically significant, probably because of the small sample size. A comparison by

an alternative statistical technique, using the standard deviation of the mean difference between surface and core (Moroney 1973), showed that the overall difference based on the averaged observations rather than a best estimate of slope is significant ($t = + 3.29$) at $P_0 \ll 0.01$. This is further evidence to support the conclusion (p. 140) that the outer layer of sausage is metabolically more active than the core because of enhanced microbial growth.

The rate of change in pH was very fast in sausages made without SO_2 (Fig. 19). Thus, in the surface sample, the rate of change in sausages with sulphur dioxide was -0.13 units/day and -0.57 units/day in the absence of the preservative. The pH obtaining on the fourth day in unsulphited sausages was lower than the pI for porcine muscle. This may account for a noticeable difference in the texture of sausages with and without SO_2, and it is tempting to suggest that the stiff consistency of sausages lacking preservative is caused by acid coagulation.

The influence of other antimicrobial agents on pH change in sausages was studied (Fig. 20). Initial rates of pH drift, calculated in the manner previously described, could be ranked (Table 45). It was notable that F + SO_2 (-0.07 units/day) and F + ASO_2 (-0.06 units/day) decreased the rate of pH drift compared

to the sulphited control (-0.10 units/day), and that fluoride (-0.24 units/day) and 2 : 4 : dinitrophenol (-0.38 units/day) significantly increased the rate ($P_0 \ll 0.01$). The changes in pH observed correlated with sensory observations on the aroma of these sausages (p. 77). Fluoride or 2 : 4 : dinitrophenol produced an acidic aroma, whereas the aroma of sausages containing F + SO_2/ F + ASO_2 was moderately stable.

The above results suggest that pH change is associated with carbohydrate metabolism. Such a view is supported by the work of Abbiss (1978) who showed a net loss in carbohydrate during storage. Additional evidence of carbohydrate involvement with pH change came from studies of sausages in which materials other than rusk were used (Fig. 21). Initial rates of change were again calculated (Table 46). The rank orders of these pH changes emphasises the contribution of glucose.

This hypothesis was further tested by adding fixed amounts of glucose to cellulose sausages containing preservative, and measuring the rate of change of pH (Table 47). A graph of rate against glucose concentration (Fig. 22) showed that above 0.1% glucose, the rate of hydrogen ion accumulation increases markedly. The addition of glucose to wrapped beef joints produces a similar effect (Shelef 1977).

TABLE 44

Averages of the combined pH values of all control sausages (Rusk + SO_2) and the significance of difference due to sample location

	Age in Days @ 22°C						
	0	1	2	3	4	5	6
Outer Surface	6.57	6.59	6.49	6.33	6.20	6.13	5.87
Core	6.54	6.60	6.46	6.38	6.39	6.36	6.21
Mean Difference	+0.03	-0.01	+0.03	-0.05	-0.19	-0.23	-1.56

Mean difference: -0.482

Number of trials: 7

"t" = +3.29

(Moroney 1973)

TABLE 45

Comparison of effects of antimicrobials on pH change, ranked by values of 't'

Antimicrobial(s)	Rate pH Change (SO_2)	Trial Rate	Correlation Coefficient	Pair N	Student's 't'
2:4:D.N.P.	-0.10	-0.38	+0.998	4	-6.26
Fluoride (F)	-0.10	-0.24	+0.999	4	-4.43
F + 2:4:D.N.P.	-0.10	-0.17	+0.997	4	-1.28
F + SO_2	-0.10	-0.07	+0.965	4	-0.38
ASO_2	-0.10	-0.16	+0.958	4	-0.30
F + ASO_2	-0.10	-0.06	+0.784	4	-0.09

TABLE 46

Comparison of effect of carbohydrates on pH, ranked by value of 't'

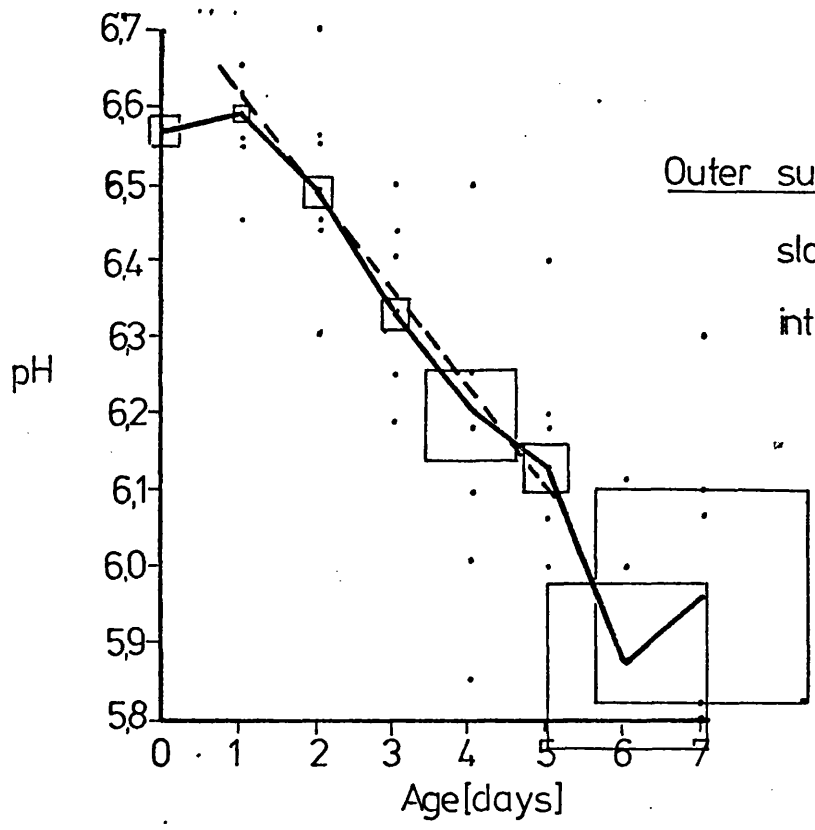
Carbohydrate	Rate pH Change (Rusk)	Trial Rate	Correlation Coefficient	Pair N	Student's 't'
Cellulose + 1.0% Glucose	-0.10	-0.80	+0.912	5	-2.96-
Flour	-0.10	-0.35	+0.982	6	-2.65
Heat-treated Flour	-0.10	-0.21	+0.982	6	=1.165
Breadcrumbs	-0.10	-0.01	+0.908	6	+0.429
Cellulose	-0.10	-0.02	+0.845	6	+0.299
Cellulose + 0.1% Glucose	-0.10	-0.14	+0.923	5	-0.180

TABLE 47

The effect of glucose on the rate of pH change (units/day)

	Glucose Concentration (%)				
	Nil	0.001%	0.01%	0.01%	1.0%
Log_{10} Concentration	-	$\bar{3}.00$	$\bar{2}.00$	$\bar{1}.00$	0.00
Surface Rate (pH/day)	-0.195	-0.18	-0.15	-0.14	-0.80
Core Rate (pH/day)	-0.105	-0.150	-0.06	-0.0	-0.03
Rusk Surface Rate (pH/day)	-0.10	-0.10	-0.10	-0.10	-0.10

Fig 18 Changes in mean pH values at two locations, at 20-22°C., with standard deviations [□] & estimates of initial rates [---] of loss, from day 1 to day 4, for sulphited sausages.



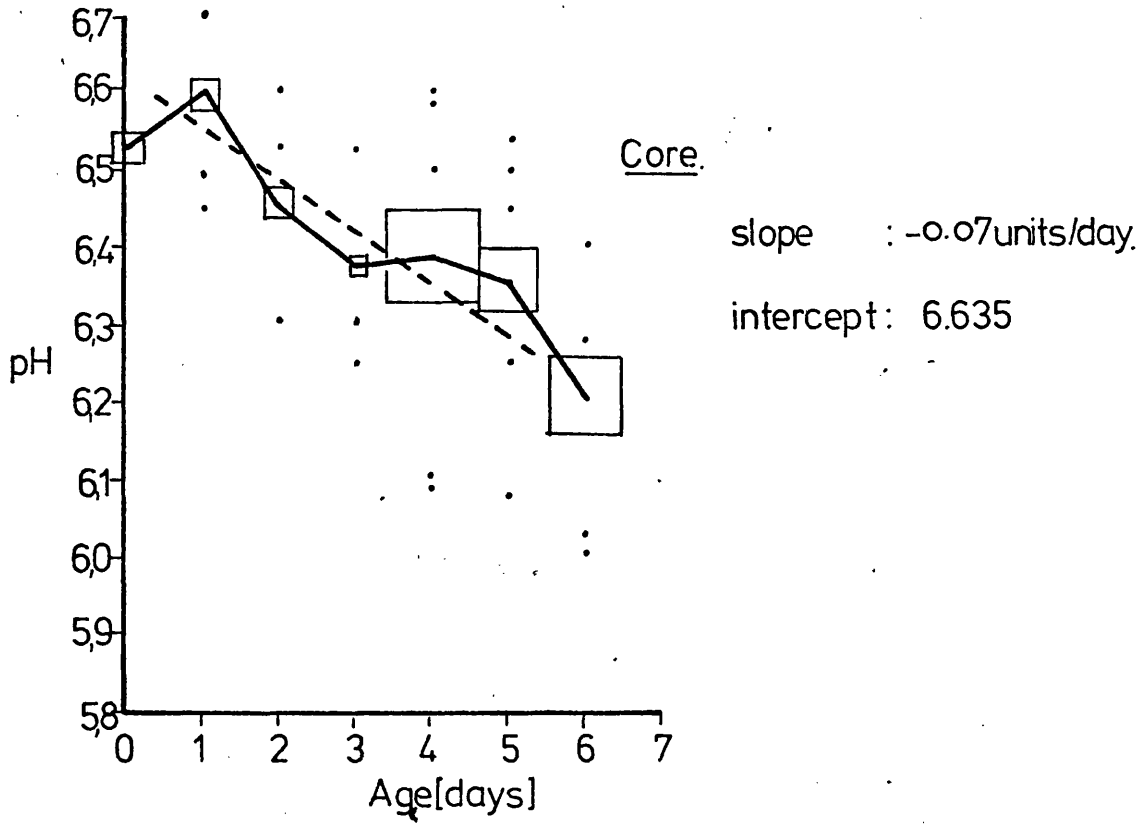


Fig 19 The effect of the presence [----] & absence [----] of SO_2 on pH at the outer surface [—] & core [----] locations.

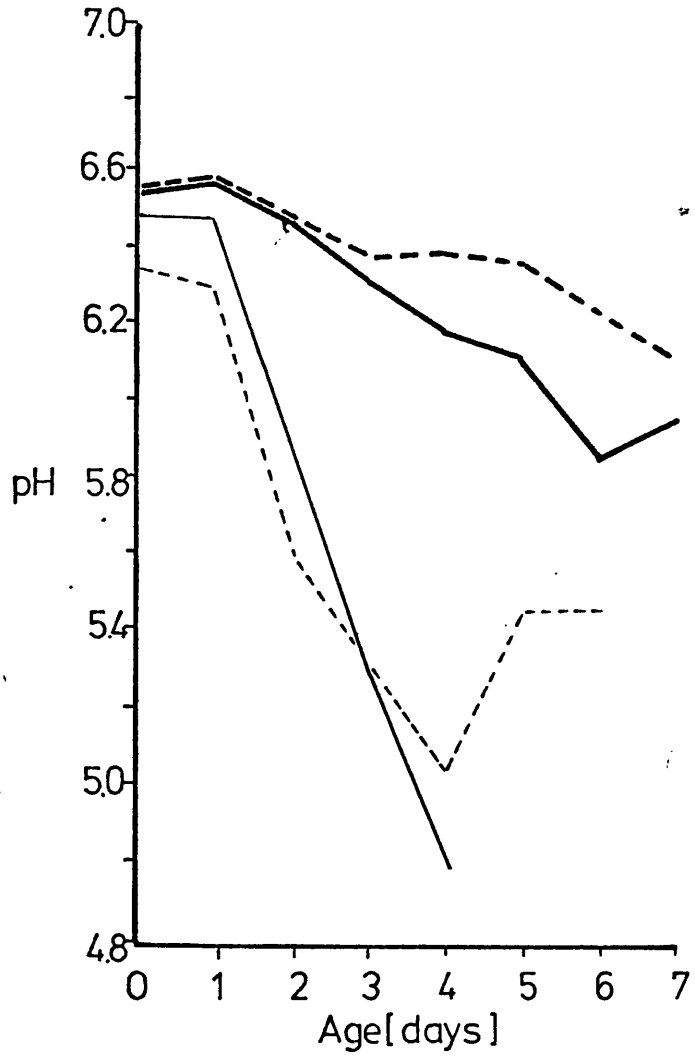


Fig 20 Effect of alternative antimicrobials on the change in pH at the outer surface location, at 20-22°C.

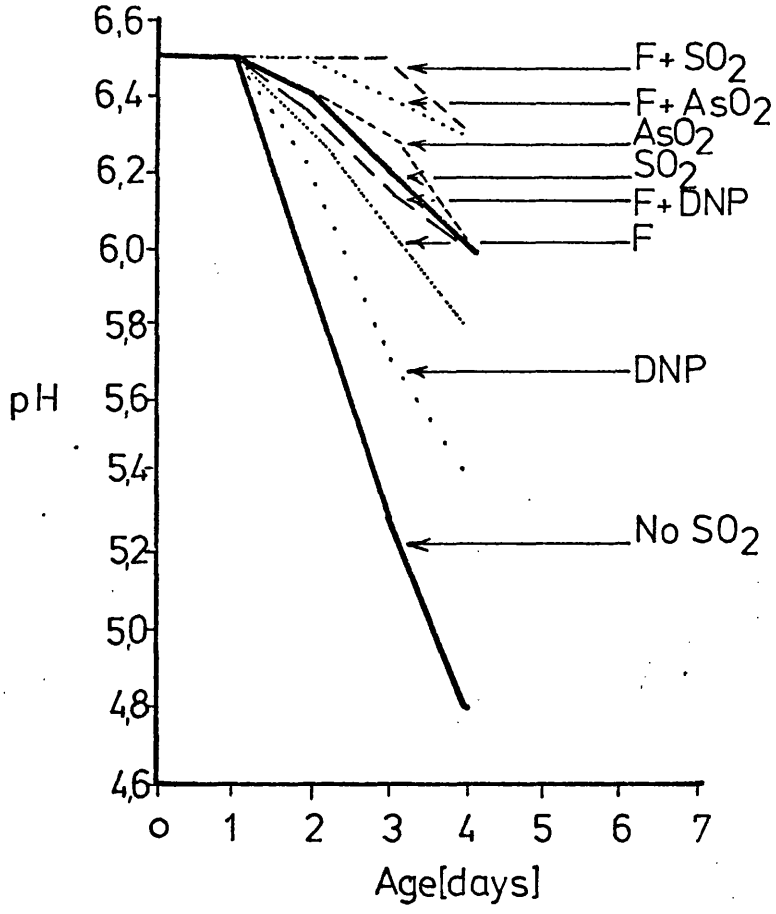
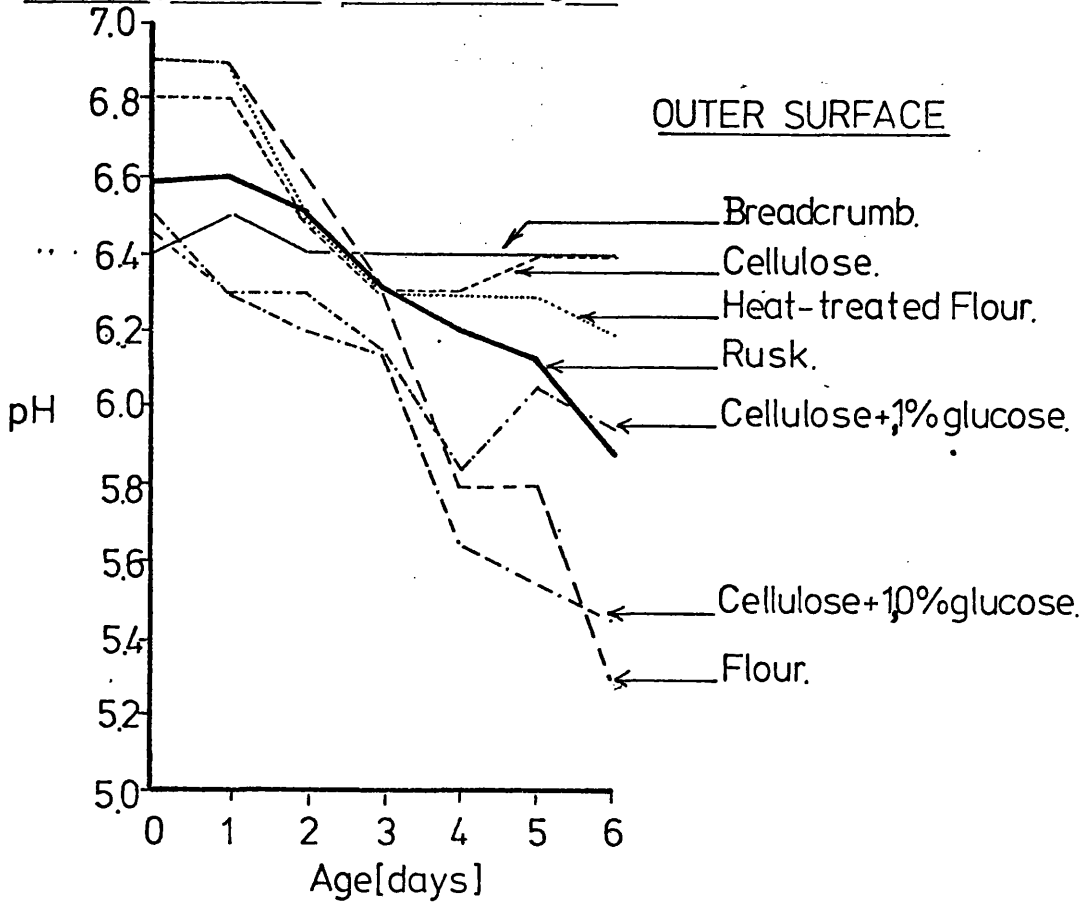


Fig 21 A comparison of the effects of rusk, & its alternatives, on the pH of sulphited sausages.



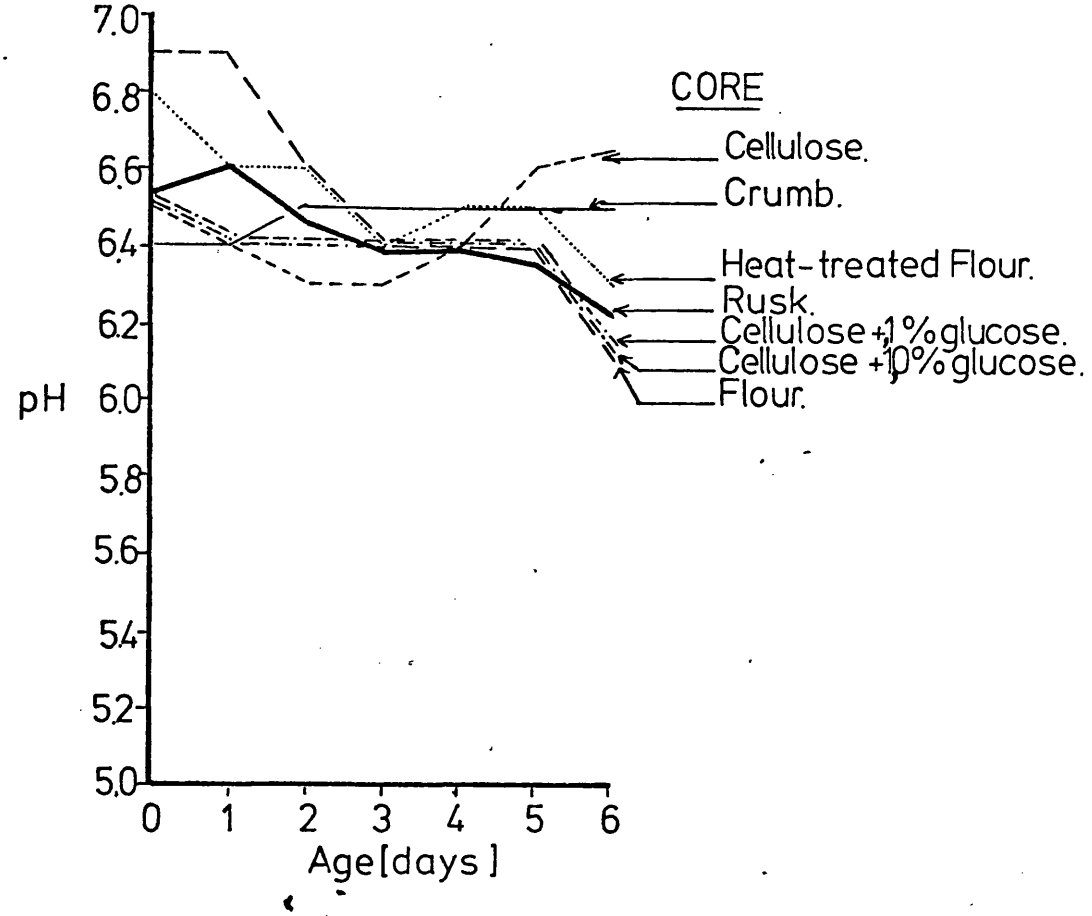
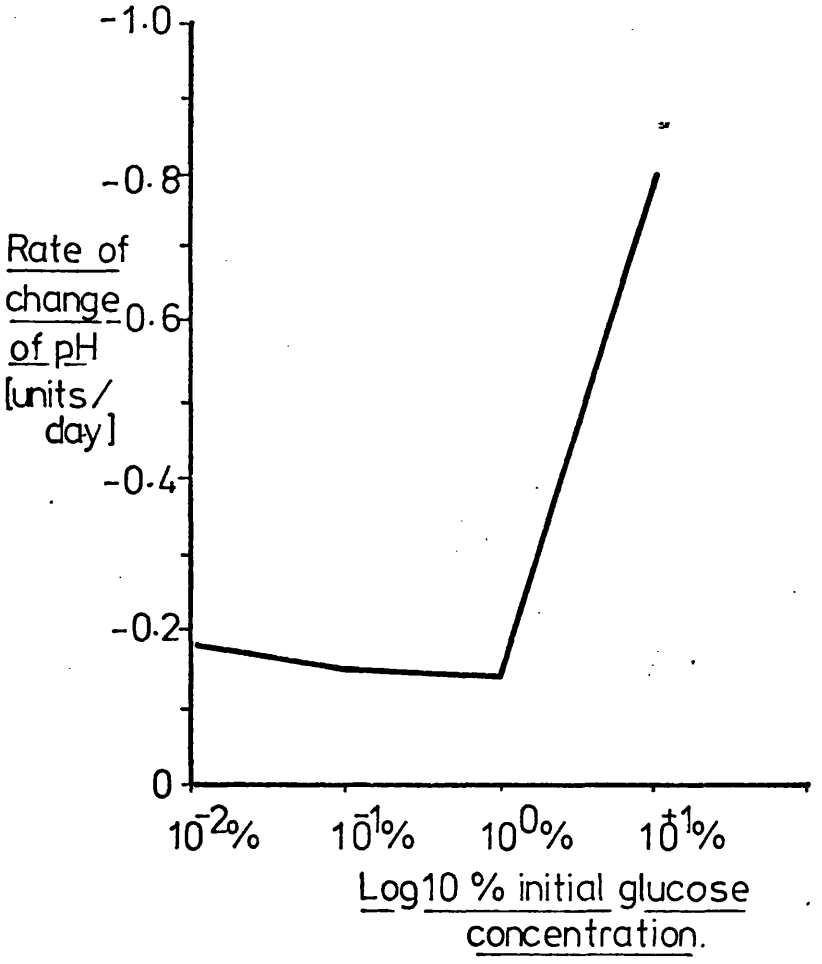


Fig 22 The effect of increasing the initial glucose concentration on the subsequent rate of change of pH [at 20 - 22°C] at the outer surface location.



(ii) The accumulation of organic acids

Typical examples of gas-chromatograms of trimethylsilyl derivatives of organic acids soluble in the aqueous phase of sausages are given (Figs. 23 and 24).

The study of organic acids produced concentrated on the outer surface and core locations, in the presence and absence of SO_2, for sausages spoiling at room temperature.

The presence of hexamethylsiloxane in the eluate rendered resolution of early peaks (e.g. acetate, butyrate, and propionate) very difficult. Three late-eluting acids could easily be resolved; lactate, valerate, and an unidentified acid, 'X'. Valeric acid was present in unsulphited sausages, but absent from sausages containing the preservative.

In the presence or absence of sulphur dioxide, lactic acid accumulated in the surface layer of sausages to approximately the same level (in arbitary units) up to the fourth day of storage. Subsequently, its production in unsulphited sausages is much greater than in the sulphited product (Fig. 25). In contrast, at the core, the lactic acid content of sulphited sausages gradually decreased with time. In the absence of SO_2, lactic acid slowly accumulated, with the rate increasing after the fourth day.

Valeric acid accumulated at both locations (Fig. 25) provided that SO_2 was not present, in the ratio 7 : 1 (surface to core).

The unidentified acid, 'X',was present in both sulphited and unsulphited sausages. In the presence of SO_2 it initially accumulated in the surface layer, but disappeared again after the fourth day. In the absence of SO_2, it only accumulates after the fourth day. In both sausage types, the level of 'X' in the core region decreased with time, but the rate and initial magnitude was greater for unsulphited products.

(iii) Metabolism of sausage fat

The presence of valeric acid in the aqueous phase of unsulphited sausages, and its absence in the sulphited product, indicated that differences in the metabolism of fat might occur between sausages containing preservative, and those made without it. The previously noted differences in organoleptic qualities, particularly aroma, also supported this view.

Preliminary studies of the fat contents of sulphited and unsulphited sausages, using thin-layer chromatography, demonstrated differences in the distribution of fatty acids between the free fatty acid 'pool' in the fat and those associated as triglycerides

(Fig. 26). Up to and including the fourth day, the rate of breakdown of triglycerides was greater in sausages which did not contain SO_2. There are points of inflection on both curves; the rate increased after the fourth day in sausages containing sulphite whereas in its absence there appeared to be a decrease.

In the presence of SO_2, there was a nett increase in the concentration of free fatty acids in the fat, which approximated to the amount lost from the triglyceride portion. After the fourth day, accumulation of free fatty acids slowed. In contrast, the size of the free fatty acid pool in unsulphited sausages remained constant, suggesting that an equilibrium existed between fatty acids entering the pool from triglyceride breakdown, and metabolism of the pool contents. These results are in accord with those of Brown (1977).

Methylated derivatives of the free fatty acids associated with glycerides and free in the 'pool' were prepared (p. 69) and changes in their distribution analysed by gas chromatography (p. 69). There were marked differences associated with the presence of SO_2. The profile of the fatty acid composition of the glycerides in sulphited sausages showed points of inflection at four and five days (Fig. 27). Similar, but opposite, changes occurred

in the profiles of the glycerides of unsulphited sausages (Fig. 28).

During the first four days of storage of sausages containing SO_2, there was loss of oleic acid (C_{18-1}) from the glycerides and an accumulation of an equivalent quantity (10-11%) in the free fatty acid pool. Similar, but smaller changes also occurred with stearic (C_{18}) and capric (C_{10}). The distribution of the other components did not change appreciably during this period.

At the fourth day of storage, when the microbial association climaxed, the rate of loss of oleic acid (C_{18-1}) from the glycerides increased and the trend towards its accumulation in the free fatty acid pool was reversed (Fig. 29). There was a concomitant increase in the pool content of the other saturated (C_{10} to C_{16}) and unsaturated (C_{18-2}, C_{18-3}) acids. From the fourth day onwards, the rate of loss of oleic acid from the glycerides accelerated, but it did not accumulate in the pool, thus suggesting a nett loss.

In the absence of SO_2, the loss of oleic acid from the glyceride portion (Fig. 28) was not associated with the accumulation of oleic. Thus, ca. 10% was released from glycerides, but only ca. 3% accumulated

in the first four days (Fig. 30).

Overall, the most significant difference observed between the two sausage systems studied lay in the change in composition of the free fatty acid portions of their fats. In the presence of SO_2, accumulation of acids, as exemplified by oleic acid, the principal component of pork fat (p. 51) occurred in the 'pool' in the same proportion as their loss from glycerides. In the absence of SO_2 the supply of free acids from esters was about the same, but only ca. 30% of the liberated acids entered the 'pool' during the first four days of storage. The remainder were metabolised, probably to valeric acid. Sulphur dioxide may therefore affect the metabolic pathway(s) responsible for the metabolism of long-chain to short-chain free fatty acids.

Fig 23

Chromatogram of TMS-derivatives of organic acids from unsulphited sausages.

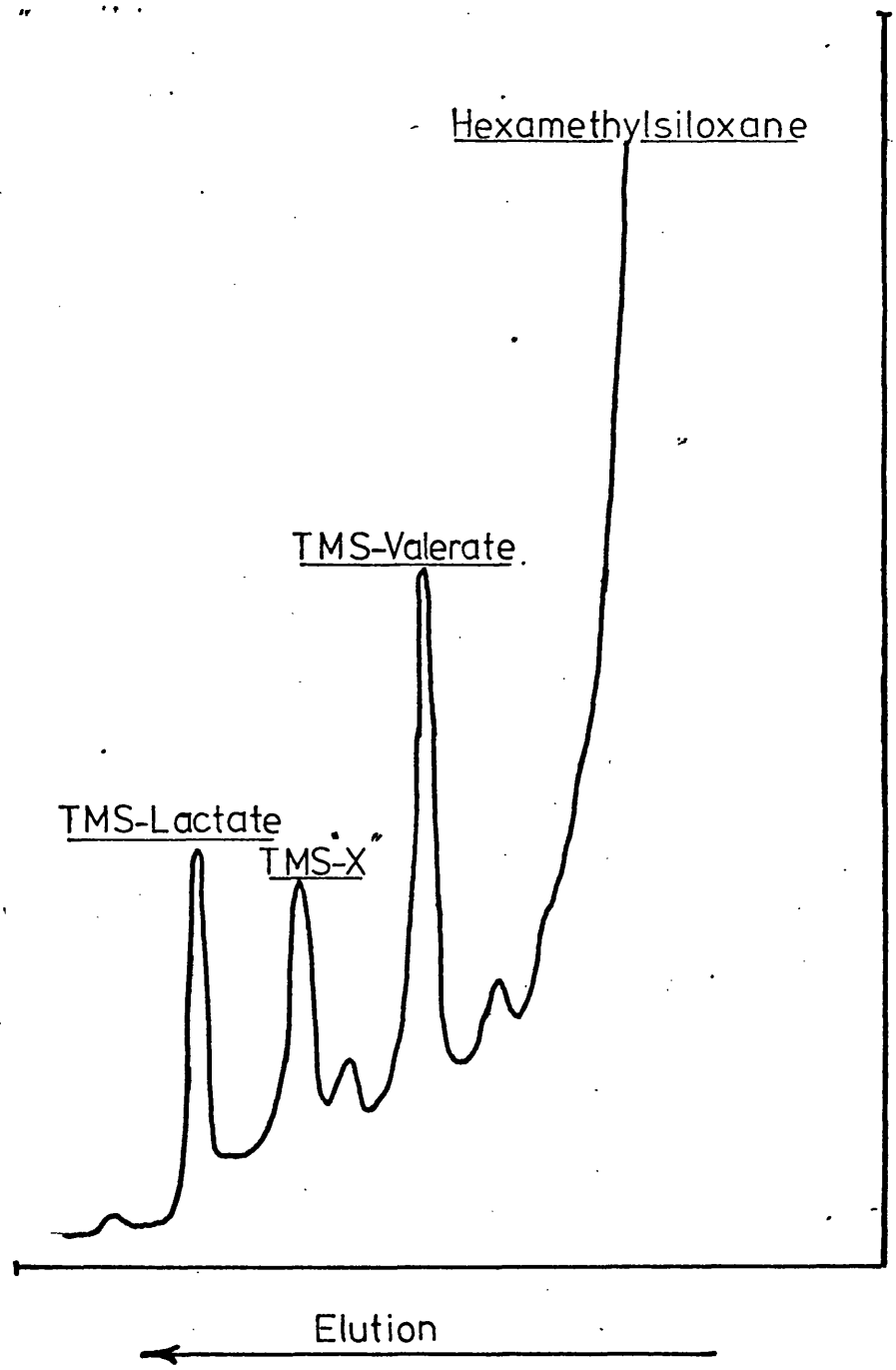


Fig 24

Chromatogram of TMS-derivatives of organic acids from sulphited sausages.

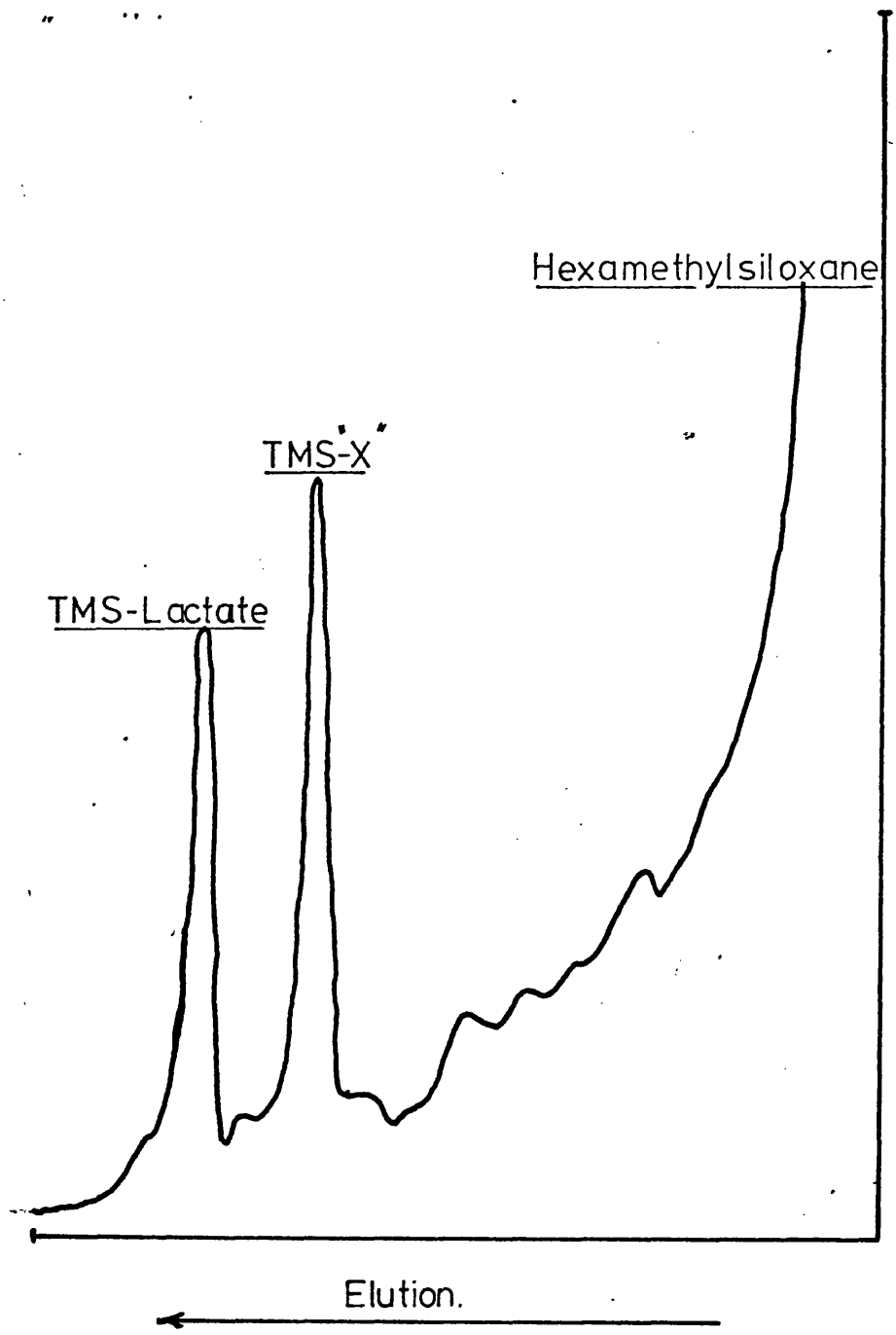


Fig 25 Effect of SO_2 on the production of organic acids [20-22°C].

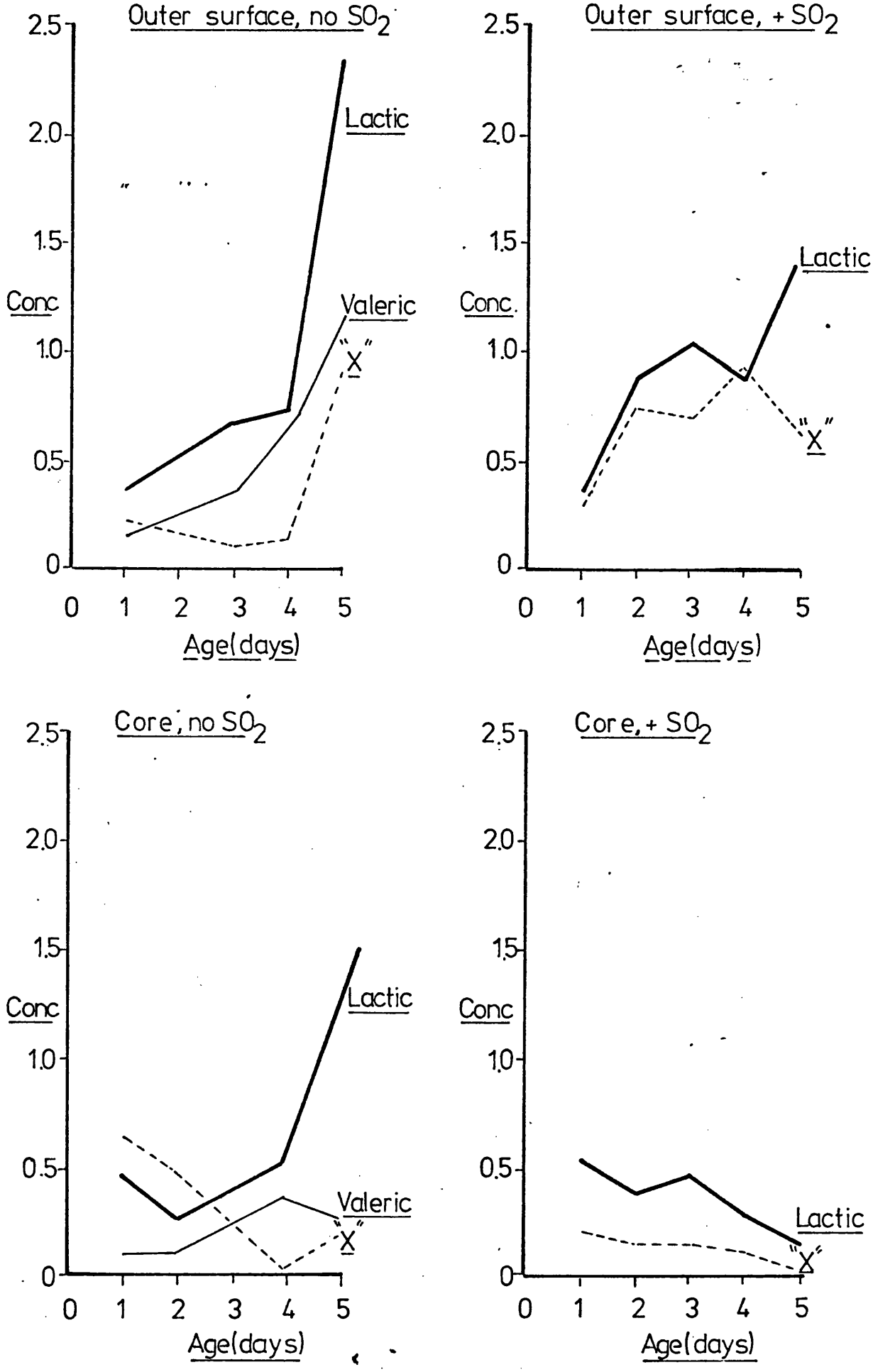


Fig 26 Comparitive changes in the proportions of triglycerides(═) & free fatty acids(┅) in the presence(—) & absence(—) of SO_2.
[outer surface , 20 - 22°C]

Fig 27 Changes in the proportions of fatty acids [as %$^{w}/_{w}$] in the glyceride portion of the fat of sulphited sausages.

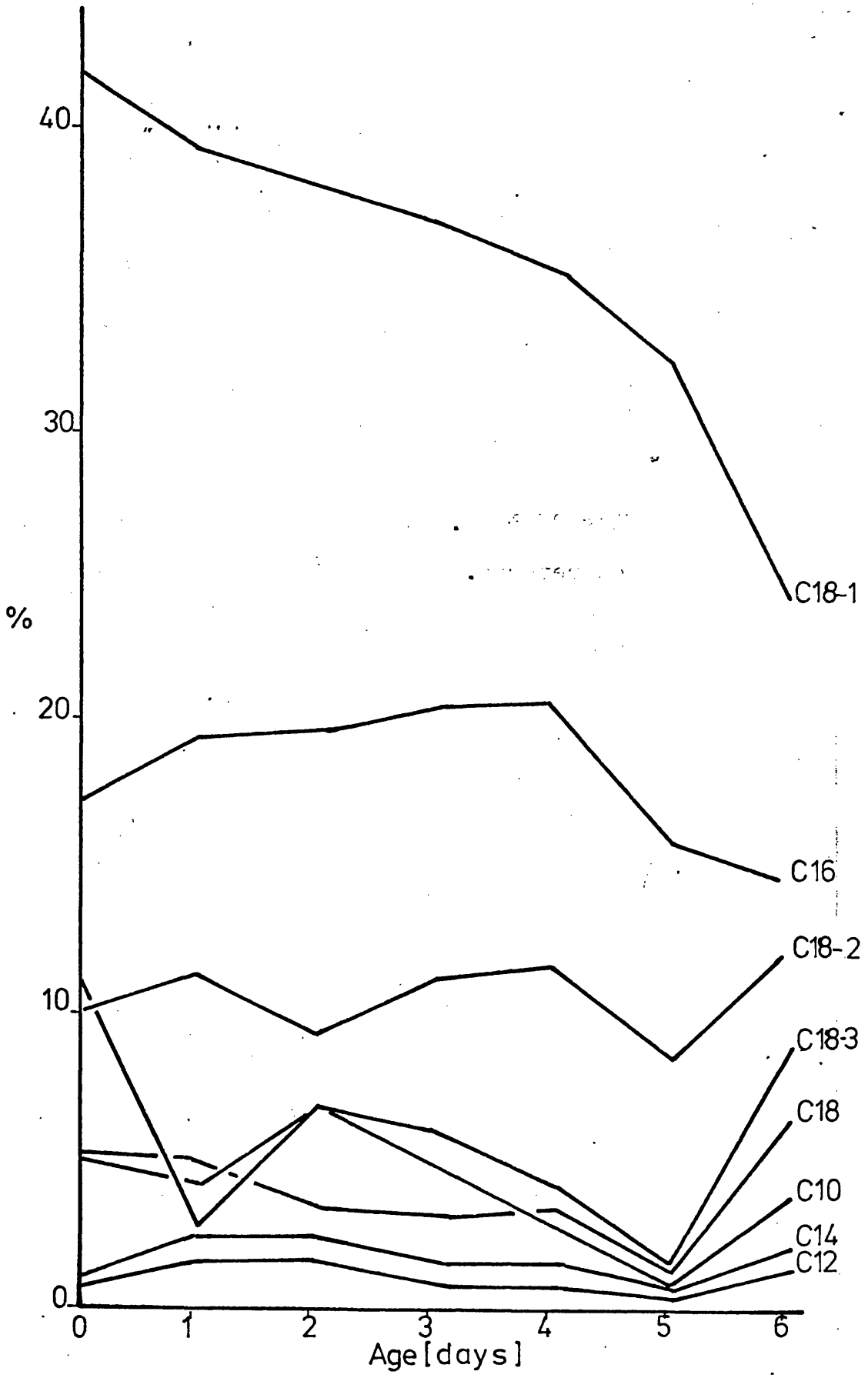


Fig 28 Changes in the proportions of free fatty acids [as % w/w] in the glyceride portion of the fat of unsulphited sausages.

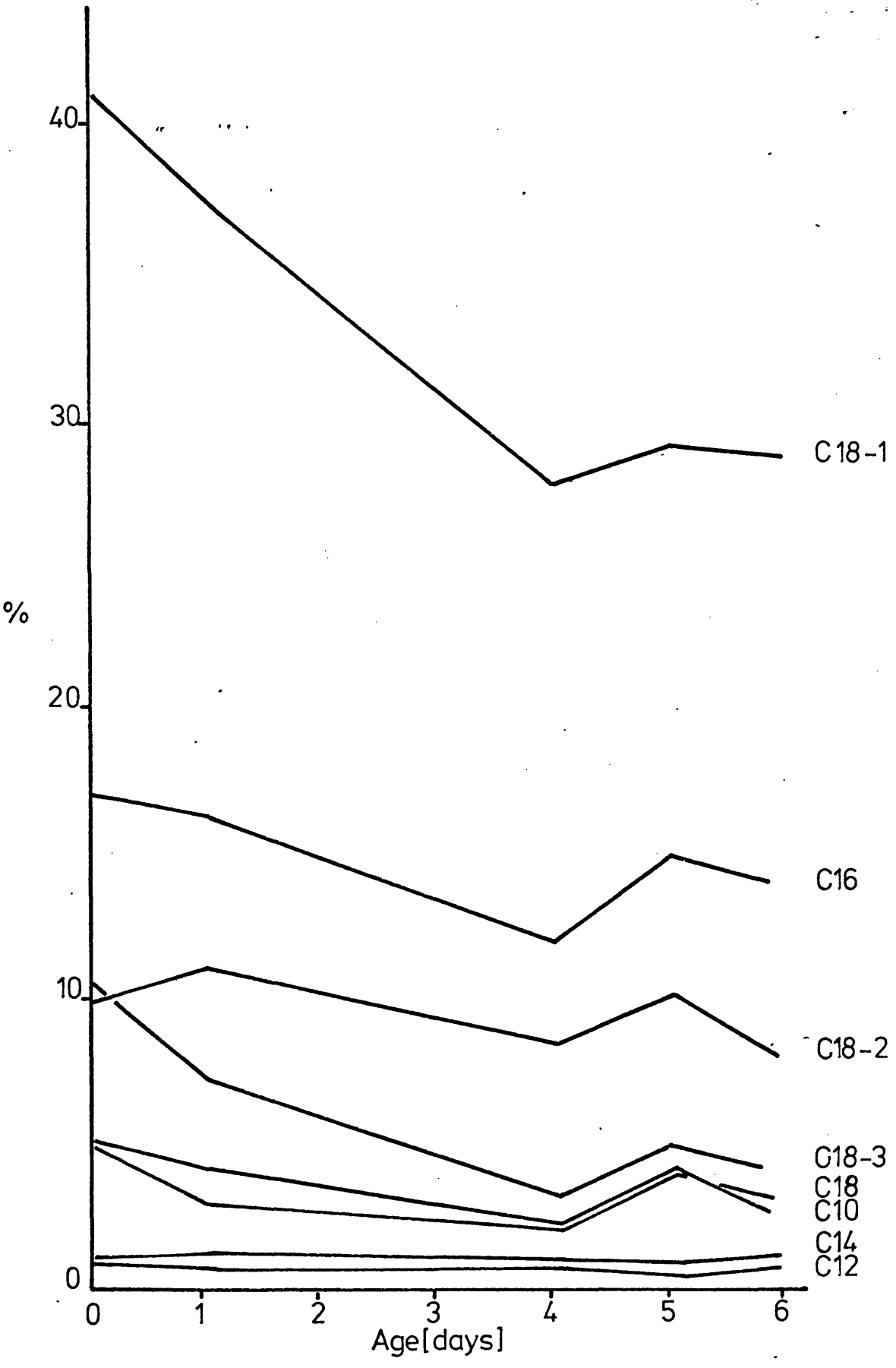


Fig 29 Changes in the proportions of fatty acids in the free fatty acid portion of the fat of sulphited sausages.

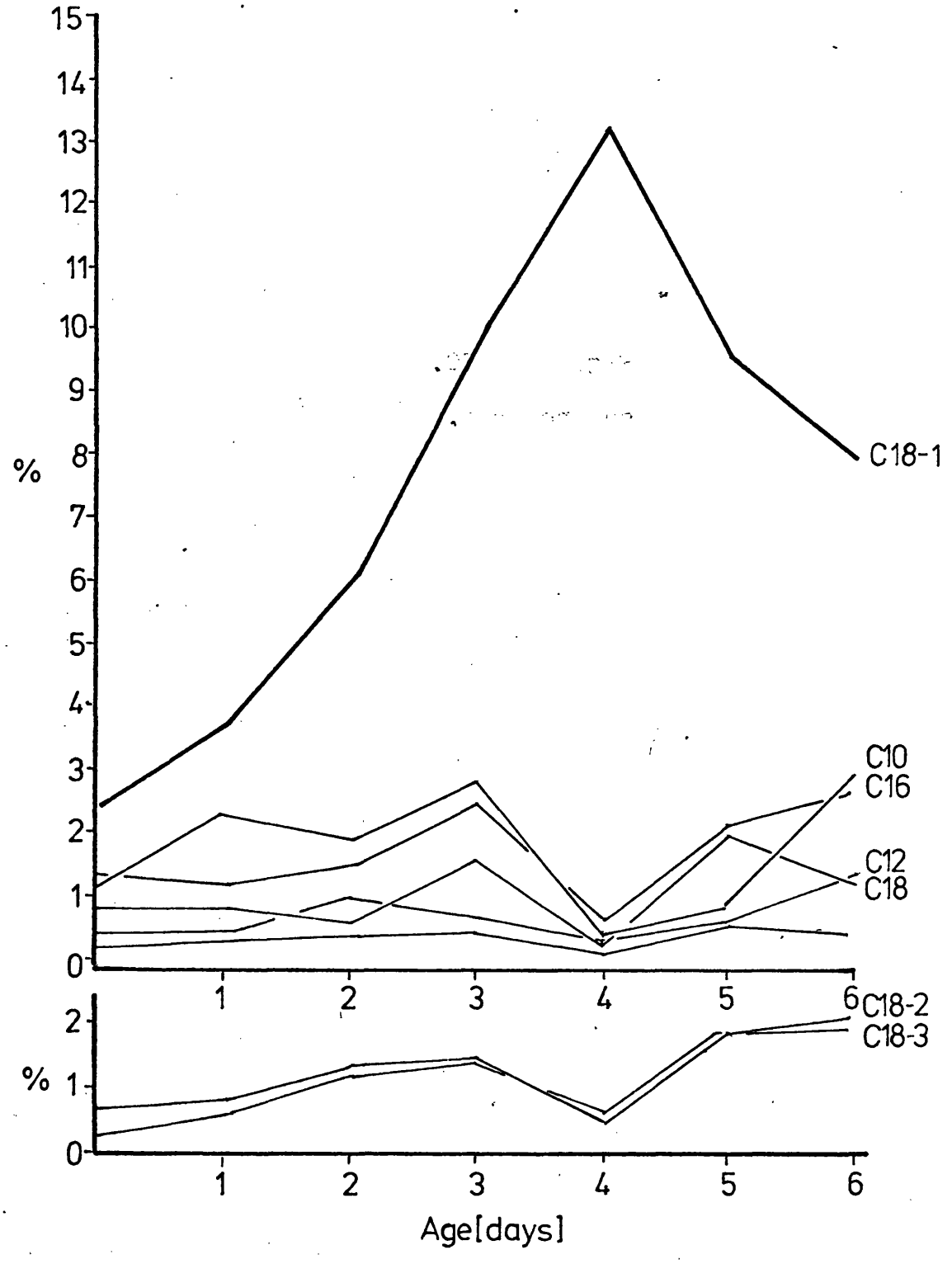
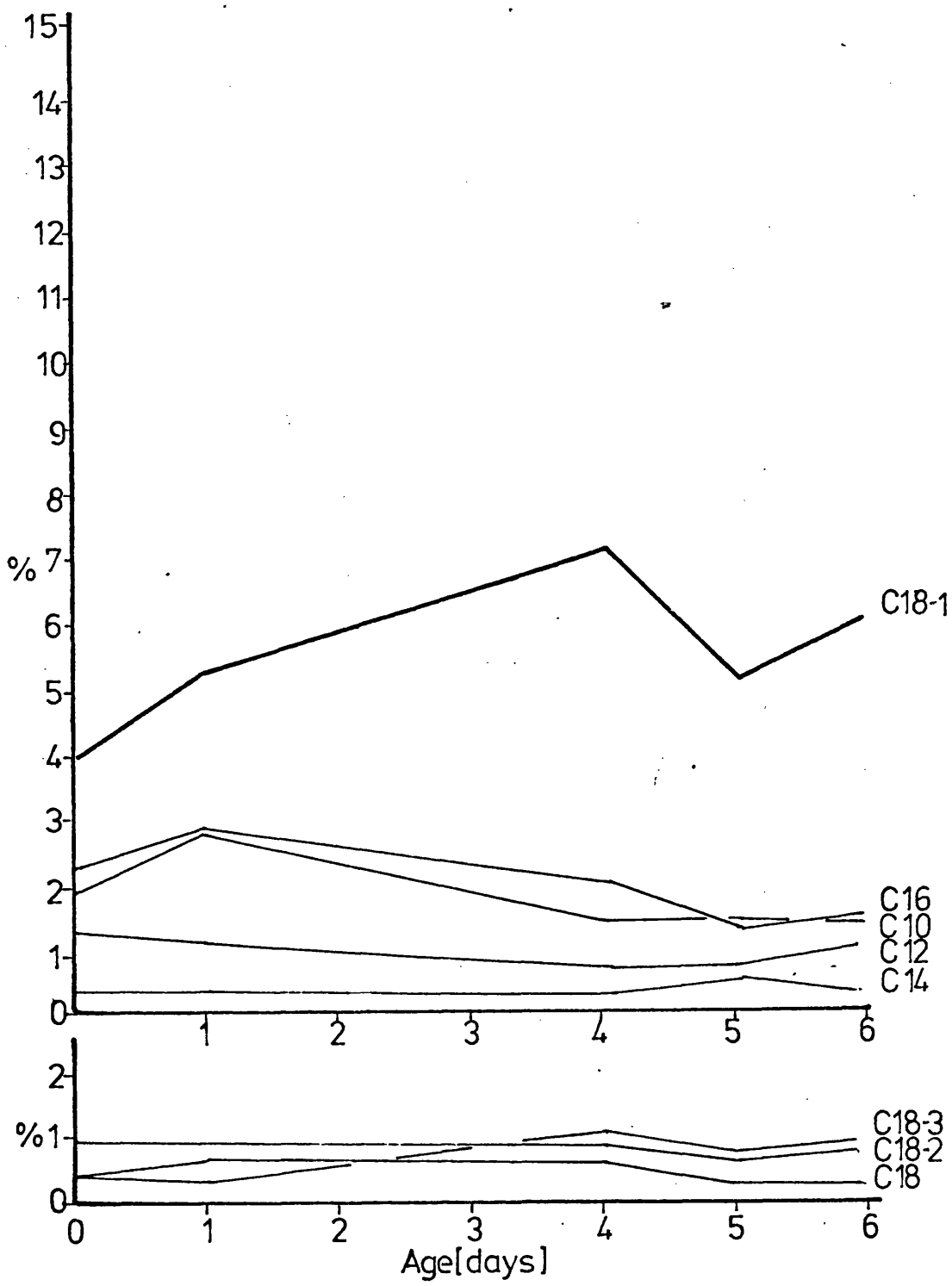


Fig 30 Changes in the proportions of fatty acids in the free fatty acid portion of the fat of unsulphited sausages.



DISCUSSIONS AND CONCLUSIONS

When considered as part of the products referred to as sausages, the British fresh variety is unique in having SO_2 and rusk as important ingredients. This study has shown that both contribute to the product's organoleptic characteristics; SO_2 determines the spoilage pattern and type of spoilage characteristics which develop, and rusk contributes to the aroma and flavour of the fresh product.

The sulphiting of sausage meat was observed to modify, both qualitatively and quantitatively, its sensory attributes during spoilage; sausages prepared without preservative changed in character within a day, and became unacceptable after two days at room temperature, darkening and becoming brown in colour (Fig. 14), and developing a cheese-like aroma (Fig. 11), and sour taste. The incorporation of 450 p./10^6. SO_2 at manufacture eliminated most of these features; the shelf-life was doubled to four days, the observed change of colour was limited to a fading from pink to a pale yellow-grey (Fig. 14) and the development of the spoiled aroma was delayed until the fourth day. The spoiled aroma lacked the cheese element, was less pungent, had a yeast-like quality and smelled fermented (p.77). Sausages in this condition were palatable (after baking in an oven) to some of the assessors on the untrained taste panel.

A comparison of the effects of a limited number of other

antimicrobials offered an explanation for these observations. In sensory terms, sulphite and arsenite produced very similar effects on the colour and aroma (Figs. 14 and 15). Arsenite, a respiratory inhibitor, was selected for its effect on dihydrolipoyl dehydrogenase enzyme complexes (Massey 1958, Massey 1960, Massey and Veeger 1961, Mahler and Cordes 1971) with subsequent inhibition of the respiration of pyruvate and α-oxoglutarate. The substitution of fluoride for SO_2 did not produce the latter's spoilage pattern; it instead mirrored the deterioration of unsulphited sausage (Fig. 15). Fluoride was selected to block glucose metabolism. Substrate concentrations of this sugar have been demonstrated in sausages by Abbiss (1978), who proposed that it served as an energy source for microbial growth. The combination of fluoride and sulphite was observed to produce the sulphite-spoilage pattern, but with a greater restriction on the rate of spoilage, particularly evident when colour retention was used as a measure (Figs. 14 and 15).

Fresh pork sausages contained raw meat. It is assumed that some of the colour changes observed are caused by changes in the oxidation state of myoglobin. The greater stability of the colour of sausages containing fluoride and sulphite (or fluoride and arsenite, which was observed to produce an identical effect) may therefore have been caused by the dual effect of a limitation for glucose as

a substrate for respiration coupled with a respiratory block. Inhibitors of respiration produce a similar effect on steaks (Robach and Costilow 1961). The sensory features of spoiled sausages blocked in this manner could not be reproduced using fluoride and 2 : 4 : dinitrophenol, an uncoupler of oxidative phosphorylation which stimulates respiration (Mahler and Cordes 1971). The effect of fluoride in the presence of sulphite or arsenite, but not in their absence, implicated glucose as a utilisable substrate for enzyme action or microbial growth in the preserved product. Further investigations, using a range of alternatives to sausage rusk (Table 12), and by adding glucose in the presence of cellulose (Table 13) demonstrated a relationship between glucose contribution and spoilage, assessed in terms of appearance (Figs. 14 and 15) and aroma (Fig. 13). Enzymically active raw flour, capable of contributing glucose because of its natural amylase content (Kent-Jones and Amos 1952) caused greater spoilage than rusk. Heat-treated flour (processed to denature the enzymes present) only promoted spoilage to a level equivalent to rusk or its fermented alternative, breadcrumb. The use of cellulose resulted in less spoilage, but this could be restored to the rusk level by adding 0.1%, and to the flour level by adding 1.0% w/w, of glucose.

The sensory changes recorded were considered to be the result of biochemical activity occurring within the

sausage, a possible vector of which was bacterial growth.

Traditionally, SO_2 has been regarded as a preservative which, when used in sausages, impeded or prevented the growth of bacteria which would otherwise cause spoilage of the product. However, Hurst (1972), Brown (1977) and Abbiss (1978) have shown that the only demonstrable anti-microbial effect of SO_2 is inhibition of the growth of coliforms. In other respects, the microbial association (p.134) is similar in composition in sulphited and un-sulphited sausages, but reduced in absolute population terms by ca. one decade in the sulphited product. A similar observation has been made in this study (Fig. 16 and overlay). A significantly greater number of bacteria were found to grow at the surface of the sausage (Table 36). A projection was also made that the maximum population possible at the surface of the sausage was $10^{8.5}$/g., and 10^{6}/g. at the core (Fig. 17). These projections approximated to the observed values for the standard product after four days of storage at room temperature (Fig. 16). The observed population differences were thought to be due to the greater availability of oxygen at the surface of the sausage. Similar observations have been made by Brown (1977) and Abbiss (1978). In addition to this limitation, microbial growth within the sausage might also be restricted by competition for available substrates with meat enzymes (p.133), a hypothesis also proposed by Brown 1977.

Varying the carbohydrate in the sausage affected the growth rate of the association, and in particular that of the dominant organism, Microbacterium thermosphactum. Raw flour accelerated, and cellulose decelerated, the latter's rate of growth when compared to rusk (Tables 39 and 40). The addition of fluoride in the presence of sulphite produced an effect comparable to cellulose (Tables 41 and 42). Abbiss's findings (1978) of substrate levels of glucose and maltose, and his views as to its putative role as an energy source for microbial growth, are confirmed by these observations. There was, however, some evidence to suggest that the glucose availability needed to be controlled for microbial growth to be maximised; the addition of glucose at manufacture to sausages containing SO_2 and cellulose showed that above 0.1% w/w added glucose, microbial growth was inhibited (Table 39). Rapid accumulation of hydrogen ions, with a resultant increase in the acid rate of pH drift, was the assigned cause (Fig. 22). Similar observations have been made for the effect of excess glucose on Pseudomonas spp. on raw beef (Sheleff 1977).

pH was used extensively in this study to monitor the accumulation of hydrogen ions and acid species (identified as lactic, valeric and an unknown 'X') in the aqueous phase. The above mentioned techniques of restricting, or conversely supplying, glucose affected hydrogen ion accumulation; where glucose was restricted in the presence of SO_2

(i.e. with fluoride, or cellulose) the pH drift recorded was less than 0.5 units. When it was made available (i.e. with flour or 1% glucose) a drift of one unit or more was observed. When SO_2 was omitted, the pH fell by 1.5 units. In this latter case, valeric, lactic and the unidentified acid accumulated, whereas in the presence of SO_2, the accumulation of valeric did not occur, and the increase in concentration of the other two was reduced by ca. 50% (Fig. 25). This is regarded as the reason for the difference in aroma between spoiled sulphited and spoiled unsulphited sausages; valeric acid has a powerful, cheese-like smell, whereas lactic acid is almost odourless.

Analysis of the fat (to investigate if it was the origin of valeric acid in unsulphited sausages) showed that the inclusion of SO_2 caused free fatty acids to accumulate, whereas in its absence their concentration remained constant. In both cases a similar decrease in the glycerol ester content of the fat (mono, di- and triglycerides) was observed (Fig. 26). The difference was inferred to be due to SO_2 preventing β-oxidation of free fatty acids during the product's shelf-life; the enzymes involved in this pathway rely on sulphydryl groups and thiol esters for their activity (Ferdinand 1978). Bisulphite (HSO_3^-), the ion species of SO_2 in solution in the sausage at the pH observed (6.5 - 6.0) (Schroeter 1966, Markris and Markakis 1974) can react with these groups and hence modify the activity of the enzymes (Ferdinand 1978), or their co-factors (Massey 1961).

The effect of SO_2 on free fatty acid accumulation was found to be particularly evident for oleic acid (Figs. 29 and 30). It is reported that this acid is more prevalent in the C_3 position of pork triglycerides, and its release is regarded as an indicator of lipase activity, probably of microbial origin (Goldman and Rayman 1951, Demeyer et al 1974). Brown (1977) has postulated that glycerol is released for catabolism (in sausages) by this means.

The continued accumulation of linoleate ceased in sulphited sausages at the fourth day of storage at room temperature, at a time coincident with the flora reaching its projected maximum, and subsequently lysing. Perceivable spoilage, sensed as a change in the aroma, also occurred at this juncture; prior to this event, this attribute had been rated as neutral and acceptable in character by the panel.

The observed variances of the three attributes rated were greatest at this neutral point on the scale, but it is recognised that this is a feature of sensory evaluation (O'Mahoney 1974). Statistically, the organoleptic quality of sulphited sausages does not significantly change until the microbial association of the surface of the sausage achieves its climax population.

The initial qualities of the three sensory attributes evaluated depended, in addition to the seasoning used, upon the type of carbohydrate incorporated in the sausage's formulation. The effect of the carbohydrates depended in

turn upon a combination of two features of their manufacture; their exposure to heat (i.e. baking) and their relative purity.

The possession of a 'baked cereal' aroma was found to be necessary in order to impart to the sausage its characteristic aroma and flavour. Unbaked carbohydrates (flour, heat-treated flour, and cellulose) lacked this quality (which was possessed by rusk and crumb) and sausages incorporating these carbohydrates resembled spiced pork.

In contrast, organoleptic stability was found to be determined by the degree of processing the carbohydrate had received. The most stable sausages were made with cellulose, the least stable with raw flour, and with rusk, crumb and heat-treated flour equal and intermediate between these two limits. Overall, rusk seemed to be the best compromise in terms of flavour contribution and organoleptic stability.

In conclusion, sausage rusk makes an important contribution to the initial aroma and flavour of fresh pork sausages. Their subsequent spoilage has been observed to occur only when the microbial association resident on the surface of the wrapped product achieves and exceeds its climax population. The relative organoleptic stability observed prior to this event is probably caused by SO_2 preventing the oxidation of sausage fats to odiferous products (e.g. valeric acid). Lactic acid is observed to accumulate, but its presence

does not seem to be related to organoleptic change. It is probably produced as a result of the metabolism of glucose produced from rusk carbohydrate by the action of meat amylases. An equilibrium is thought to have been struck - empirically - between the need to supply glucose in order to keep the flora active (essential for organoleptic stability in the presence of sulphite, in. that once the flora dies, the organoleptic qualities of the sausage become unacceptable) and the need to limit the rate of supply of glucose to that which is fulfilled by meat amylases, and so prevent too rapid growth of the flora, and an early climax, with a concomitant shortening of the shelf-life (as is produced by excess glucose production due to the action of flour amylases).

COMMERCIAL IMPLICATIONS

It would appear, from the results discussed so far and those of Brown (1977) and Abbiss (1978) that the British fresh pork sausage will be difficult to improve (from a storage point of view) without disturbing the equilibrium which seems to have evolved naturally. For example, the use of raw materials of good microbiological quality, with the aim of giving a low initial total count, might not produce appreciable gains in shelf-life; the growth rate of yeasts under these conditions at the outset has been shown in this study to be close to the theoretical maximum calculated for organisms growing in sausages, and will be sufficient to give them early dominance of the flora. Their greater metabolic activity will compensate for their smaller initial population numbers, and it is likely that the sausage will be regarded as having spoiled within the same time scale as would normally be observed for a bacterially spoiled product. Although sausage rusk has been identified as the source of glucose (Abbiss 1978), the fermentable substrate utilised by the flora in the presence of sulphite, it is important to the organoleptic quality of sausages because it is a baked product. Its principal commercial competitor, breadcrumb, can make a similar contribution, but breadcrumb is more expensive to manufacture. Cheaper alternatives - for example, flours and heat-treated flours - cannot make this contribution to the development of the flavour, do not complement the spices also added as a seasoning, and

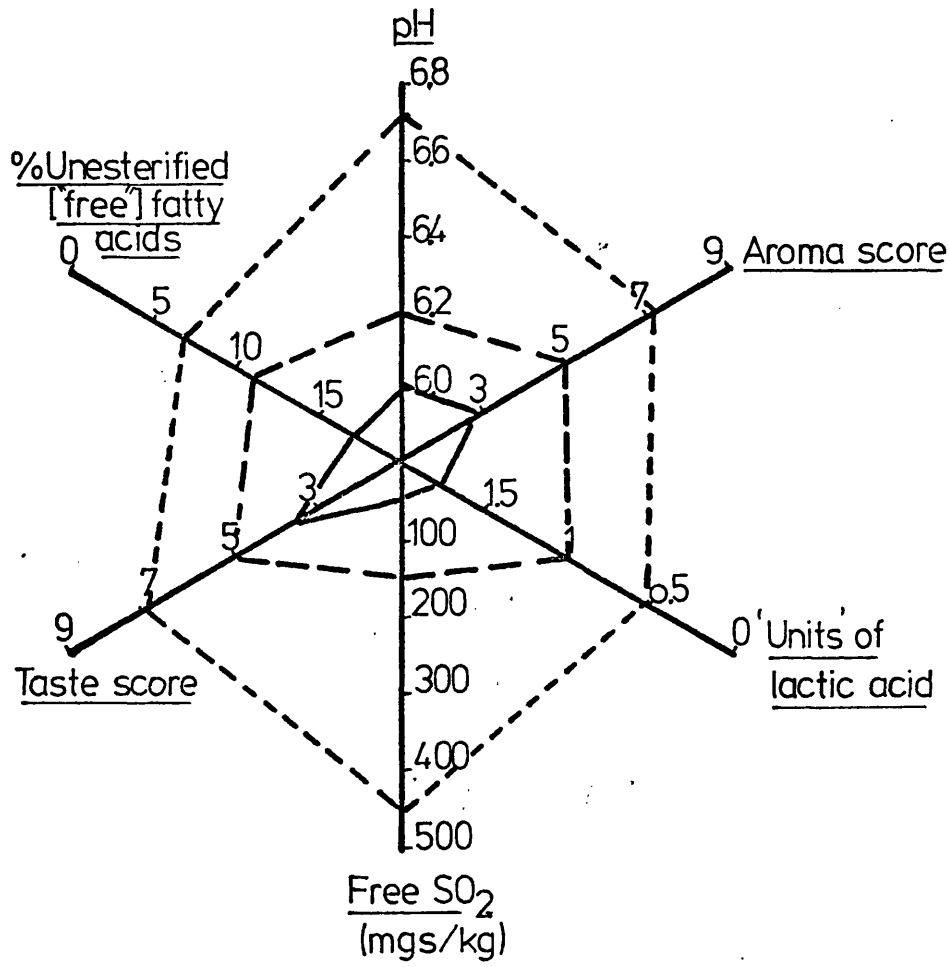
can also (in the case of flour) stimulate the growth of the microbial association, thereby enabling the climax population to be achieved earlier, and the product's shelf-life to be shortened. Rusk, from the viewpoint of cost, water absorption, and aroma and flavour contribution, is probably the best farinaceous product for use in British fresh sausages in their current form. Few acceptable alternatives exist.

SO_2 appears to be essential to the microbiologial safety of sausages in addition to their organoleptic stability. The omission of the preservative permits the growth of coliform organisms (Hurst 1972, Brown 1977, Abbiss 1978), in addition to permitting the development of an objectionable aroma and high lactic and valeric acid concentrations. Its role would not, therefore, appear to be strictly antimicrobial, and consequently its replacement (e.g. if necessitated by harmonization with E.E.C. requirements) might be very difficult to achieve.

The sausages studied in this thesis seemed to deteriorate quickly in step with the microbial association achieving its pre-climax peak population of ca. 10^8 c.f.u./g. Before this event, the aroma and colour of the product do not change appreciably from their initial 'bland' character, and are not unacceptable. Other biochemical features: pH, pX_{SO_2}, free fatty acid content, and lactic acid content, do change. A six axis plot, similar to the Q.D.A. diagram

(Quantitative Discrimination Analysis) of Mecredy, Sonneman and Lehmann (1974) has been constructed, for a typical pork sausage at three stages in its shelf-life (Fig. 31). It is proposed that this technique could be used for assessing changes in the quality of fresh sausages during storage, or for comparing the organoleptic qualities of different formulations. By constructing the plots on acetate transparencies and using an overlay technique, comparisons may easily be made. The areas within the boundaries can also be quantified (e.g. using a planimeter, or gravimetrically) and this could be useful for quantifying improvements in formulations by allowing statistical analysis of the results.

Fig 31 A six-axis plot of organoleptic & sensory-related biochemical variables applicable to sausage quality control.



Boundary typical of fresh sausages.

Boundary of sausages at limit of shelf-life.

Boundary of spoiled sausages.

APPENDIX I

Comparative analyses of flours of different extraction rates

	Moistures(%)	Invert sugar(%)	Starch(%)	Protein(%)	Fat(%)
100%	15	Trace	73.4	8.9	2.2
85%	15	Trace	79.1	8.6	1.5
80%	15	Trace	80.8	8.2	1.3
75%	15	Trace	81.5	8.0	1.1
70%*	15	Trace	81.9	7.9	1.0

Source: McCance and Widdowson (1960)

* The grade used for sausage rusk

APPENDIX II

Examples of microbiological qualities of fumigated herbs and spices

Herb or Spice	TVC/g.	Spores/g.	Yeasts and Moulds
Ground Nutmegs	$10^2 - 10^3$	$10^2 - 10^3$	$10^1 - 10^2$
Ground Mace	$10^2 - 10^3$	$10^2 - 10^3$	$10^1 - 10^2$
Ground black Peppers	$10^3 - 10^6$	$10^2 - 10^5$	$10^2 - 10^3$
Ground white Peppers	$10^2 - 10^3$	$10^2 - 10^3$	$10^1 - 10^2$
Ground Coriander	$10^3 - 10^5$m	$10^3 - 10^5$	$10^1 - 10^2$

Source: T. Lucas microbiology department records

APPENDIX III

Examples of microbiological qualities of soya protein; water; fat emulsions.

Examples	TVC/g.	Coliforms/g.
A	9.65×10^6	3.2×10^6
B	5.25×10^7	5.0×10^7
C	4.0×10^4	2.0×10^4
D	1.28×10^6	1.8×10^6
E	3.32×10^6	7.6×10^5

Source: T. Lucas microbiology department records

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